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THE PHILIPPINE JOURNAL OF SCIENCE

PAUL C. FREER, M. D., PH. D.
GENERAL EDITOR

SECTION B

THE PHILIPPINE JOURNAL OF TROPICAL MEDICINE

RICHARD P. STRONG, PH. B., M. D.
EDITOR

MANILA
BUREAU OF PRINTING
1912

PUBLICATIONS FOR SALE BY THE BUREAU OF SCIENCE, MANILA, PHILIPPINE ISLANDS

REPORT OF THE INTERNATIONAL PLAGUE CONFERENCE.

Held at Mukden, April, 1911, under the auspices of the Chinese Government.

Edited by Erich Martini, G. F. Petrie, Arthur Stanley, and Richard P. Strong.

483 pages, 18 plates (2 colored, 4 half-tones, 12 charts and maps).

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By Herbert S. Walker.

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THE PHILIPPINE JOURNAL OF SCIENCE

PAUL C. FREER, M. D., PH. D.

SUCCEEDED BY

ALVIN J. COX, M. A., PH. D.
GENERAL EDITOR

SECTION B

THE PHILIPPINE JOURNAL OF TROPICAL MEDICINE

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THE PHILIPPINE
JOURNAL OF SCIENCE
B. THE PHILIPPINE JOURNAL OF
TROPICAL MEDICINE

VOL. VII FEBRUARY, 1912 No. 1

THE RESULT OF THE PAST TWO YEARS' WORK IN THE STUDY OF TROPICAL SUNLIGHT.

By PAUL C. FREER.

(From the Bureau of Science, Manila, P. I.)

Two years ago, I outlined some of the problems to be solved so as to give us an intelligent appreciation of the influence of different intensities of insolation, in various parts of the globe, upon the inhabitants thereof. I then pointed out that, perhaps, the first work should be comparative measurements, undertaken during reasonably long periods, in different latitudes, of the effect produced by that section of the spectrum of the sun to which, in the major portion of the literature, the greatest effect is generally ascribed, namely, the rays of greater refrangibility in the violet and ultra-violet. In suggesting this line of investigation, I did not lose sight of the fact that measurements of the total insolation and of the effect of other portions of the spectrum might be even of greater importance.

It is necessary to resort to a photocatalytic reaction to obtain data regarding the relative influence of the rays of shorter wave length on different days and in different latitudes, and therefore members of the staff of the Bureau of Science set themselves the task of investigating and making available the photocatalysis of oxalic acid in the presence of uranyl salts, as, in the decomposition in question under the normal conditions of the reaction, there is no reduction of the uranium compound and apparently there are no side reactions to complicate the conclusions.

The decomposition of oxalic acid into carbon monoxide (respectively formic acid), carbon dioxide, and water is brought about almost entirely by those rays in the spectrum of the sun extending from 550 $\mu\mu$ to 291 $\mu\mu$. The sun's spectrum does not extend below the latter point, even in the Tropics.

That the decomposition is due to these rays is shown by the following summary of data obtained by exposing standard solutions of oxalic acid and uranyl acetate[1] in equal sized (standardized) vessels to the action of the sun during equal periods of time, the vessels being covered with various U-viol glasses, as follows: (1) Without cover; (2) with U-viol 280 $\mu\mu$; (3) U-viol 289 $\mu\mu$; (4) blue U-viol No. 3653; (5) U-viol copper-ruby No. 2745, all the glasses being 5 millimeters thick. The decomposition in number 1 being placed at 1, those taking place in equal times in the others were:

Number 2 (U-viol 280)	0.888
Number 3 (U-viol 289)	0.883
Number 4 (Blue U-viol No. 3653)	0.508
Number 5 (U-viol copper-ruby No. 2745)	0.004

The ultra-violet glasses, with the limit slightly beyond the range of the sun's spectrum, exert some absorptive influence; this rapidly increases to the blue, and practical extinction occurs before the red. Similar results were obtained by Bruner and Kozak,[2] who filtered the sunlight through a cold, saturated solution of potassium dichromate of 2 centimeters thickness, and thereby brought the reaction to a standstill. The absorption bands of uranyl salt solutions, according to Deussen,[3] terminate at 487.5 $\mu\mu$, but the decomposition *by light* of our standard solution extends below this point, as the cobalt-blue practically terminates at 490 $\mu\mu$ and we had minimal decomposition in the red at about 650 $\mu\mu$. However, as we used no glasses between the cobalt-blue and ruby-red, it seems probable that the action of light in the uranyl acetate-oxalic acid solution practically terminates at about 530 $\mu\mu$, in the green.

Therefore, so far as the range of its absorption spectrum is concerned, we have in uranyl acetate-oxalic acid a most satisfactory photocatalytic reagent for carrying on the comparative measurements in question. However, although the light rays accelerating the reaction may not extend below, say 530 $\mu\mu$, nevertheless, there may be an appreciable heat factor influencing the reaction and due to other causes.

Bacon,[4] when he first studied the reaction, concluded from two experiments, in which he exposed solutions of uranyl acetate and oxalic acid, at 30° and 100° respectively, simultaneously to the sunlight, that no temperature coefficient existed over a wide range of temperature, and Bruner

[1] Five cubic centimeters of a 1 per cent solution of uranyl acetate, 5 cubic centimeters of a 10 per cent oxalic acid solution (crystallized), and 20 cubic centimeters of water.

[2] *Ztschr. f. Elektrochemie* (1911), 17, 358.

[3] *Ann. d. Phys. u. Chem.*, (Wiedemann) (1898), 66, 1128.

[4] *This Journal, Sec. A* (1910), 5, 290.

and Kozak,[5] in a range of temperature observations extending from 4° to 80°, using uranyl nitrate and oxalic acid, could observe no acceleration with increasing temperature, and observe "this is probably the first photochemical reaction with such a very small temperature coefficient, if, indeed, the latter is not equal to naught." The mixture, heated in a water-bath in the dark, shows no decomposition, and flasks containing uranyl acetate-oxalic acid solutions, when covered with thin silver foil and exposed to the full effect of the sunlight, likewise show no change even though they become markedly heated.

Therefore, it seemed safe to assume that a temperature coefficient would be negligible or nonexistent in comparative measurements, but we were once more brought back to this phase of the reaction by an observation made by Mr. M. Barrowcliff, of the Institute for Medical Research at Kuala Lumpur,[6] who, in carrying on measurements with the standard solution, called our attention to the fact that, in his opinion, a temperature coefficient in reality existed.

The entire matter was open for reinvestigation, and Mr. W. C. Holmes, of the laboratory of organic chemistry of the Bureau of Science, conducted a careful series of experiments confirming Mr. Barrowcliff's results. There is very little difference between the reaction at 30° and at the boiling point, as Bacon had shown, but between 2° and 30° there is a marked increase in the rate of decomposition with the rise of temperature, so much so, that decomposition at 2° is only 60 per cent of that at 30°, whereas that at 30° is 95 per cent of the decomposition at 75°; above this point, there is even a diminution of the rate with increased temperature. Between ordinary points of measurement in our climate, say from 25° to 35°, the temperature coefficient may, therefore, be neglected; where measurements at lower temperatures are made, the correction would need to be applied, although in comparing tropical climates with those of temperate zones this temperature coefficient would work in the direction of greater contrast and apparently lesser insolation in the latter, so that, if such contrast were *not* evident on comparing measurements, the coefficient could be neglected in drawing comparative conclusions. Wherein the difference between these results and those obtained by Bruner and Kozak lies, we have not yet determined.[7]

Other factors influencing the reaction must also be considered before the method can be used in a series of comparative measurements; these are: The nature of the background and the size

[5] *Loc. cit.*, 357.

[6] Communicated by letter.

[7] Possibly, in the dilute solutions we use, the temperature coefficient of the decomposition of oxalic acid alone becomes evident. It scarcely seems practical to use greater concentrations of uranyl acetate in cold climates.

and character of the flasks used. Uranyl acetate-oxalic acid solution exposed on a surface of glazed black paper was decomposed in a ratio of 0.73 to 1 for glazed white paper and 0.74 for a black, dull background. Although there is practically no difference between a glazed black or a dull black background, it is better to adhere to one kind; therefore, the dull paper was selected.

A much greater variation is brought about by the materials of the flasks used, as well as by their size and shape. Obviously, a quartz flask is best, as it allows the ultra-violet rays to pass with a minimum of absorption, but the difference in absorption between quartz and Jena glass is not so great as might be supposed. Of greater influence is the size and shape of the vessel, that is, of the surface of liquid exposed. Bacon [8] has already pointed out that the rate of decomposition increases with the size of the flask; but even between flasks of the same capacity a variation is found which can readily be understood when we realize that two 100-cubic-centimeter Erlenmeyer flasks of Jena glass may differ 50 per cent in weight. Two such vessels, of equal capacity, of 100 cubic centimeters or under do not vary more than 2 per cent, and standardized flasks were used in our measurements, wherever possible.

Bacon [9] showed that the concentration of oxalic acid, excepting at great dilution, does not influence that speed of the reaction, and Bruner and Kozak have confirmed this result. Obviously, when decomposition of the acid reaches a point where its concentration has diminished below the critical one, the reaction will gradually diminish in rate; however, this point, in the solutions used for comparative measurements is not reached until more than 60 per cent of the acid has been decomposed, a number rarely reached in three-hour exposures, and even then the initial diminution is small, but the fact must be taken into consideration where longer exposures are resorted to.

To sum up. The decomposition of a solution of uranyl acetate-oxalic acid by the sunlight is by no means a perfect indicator, for comparative purposes, of the total ultra-violet photocatalytic effect of the sunlight, for it suffers from the errors outlined above; but when the nature of the measurements is taken into consideration and when we consider that the object to be attained is a knowledge of the average influence of the sunlight during long periods in various latitudes, small individual errors can be

[8] *Loc. cit.*, 288. [9] *Loc. cit.*, 285.

neglected. The most serious difficulty is the temperature coefficient, which would become apparent in colder climates, thus bringing down the average during the winter months, but where the temperature is known this can be compensated for by calculation; the flasks can be calibrated, the exposure made as far as possible from disturbing influences, and so, comparative measurements conducted with a sufficient degree of accuracy to give us a relative knowledge of the total influence of the rays of the sun in the more refrangible portions of the spectrum. If there are great contrasts between various regions, they will be apparent despite any errors in the method.

The basis of investigation having been determined, in addition to arranging for the carrying on of daily observations in Manila, I asked colleagues in various parts of the world to coöperate by a series of measurements with calibrated flasks and standard solutions exposed on a dull black surface, free from buildings, between the hours of 9 and 12. Returns from all the places coöperating are not yet at hand, but, so far results [10] can be reported from Kuala Lumpur [11] (latitude 3° 10′ north); Honolulu [12] (latitude 21° 18′ north); Washington [13] (latitude 38° 59′ north); Tucson, Arizona [14] (latitude 32° 12′ north) and Khartoum, Egypt [15] (latitude 15° 36′ north).

In Manila (Table I) the average per cent of oxalic acid decomposed for one hour during one year was 12.45, with a maximum of 17.8 for the highest observed day and a minimum of 1.15. The average of all days *above* the general mean was 14.65, and below 9.64. Strange to say, the lower average in Manila did not fall during the rainy months of July to October, but occurred in November, and the clear months of January, February,

[10] The figures presented in this paper are for some of the places subject to reinvestigation with standardized flasks and solutions, but, in time, as the work progresses, a large range of latitudes will be covered by exact comparative measurements.

[11] Through the kindness of the Institute for Medical Research, Dr. Henry M. Fraser, director, Mr. M. Barrowcliff making the titrations; a quartz flask was used.

[12] Through the kindness of the Hawaiian Agricultural Experiment Station, Dr. E. V. Wilcox, in charge, Mr. W. T. McGeorge making the titrations, using a 200-cubic-centimeter Erlenmeyer flask which has been sent to this laboratory for standardization since the above was written.

[13] Through the kindness of Dr. Raymond F. Bacon, Bureau of Chemistry.

[14] Through the kindness of Dr. H. Spoehr, Desert Laboratory.

[15] Through the kindness of the Wellcome Research Laboratories, Dr. Andrew Balfour, director, Dr. W. Beam, chemist.

and March do not show as high a figure as the comparatively cloudy ones of June and July.

Kuala Lumpur (Table II) shows a slightly higher average, 15.29 as against 12.45, but its maximum is somewhat higher (18.1 against 17.8) and its minimum much higher, namely 9.0 as against 1.15 for Manila. In other words, the insolation in regard to the rays under discussion in Kuala Lumpur on average clear days is practically the same as in Manila, but the cloudy and hazy weather of our island climate shuts off such a proportion of the sunlight that the total effect is that of a climate having less insolation; in other words, the difference between two places, one practically on the equator and the other 14° north is a meteorological one, and not due to any excess *per se* of the shorter wave lengths in the former.

Honolulu (Table III) shows an average of 13.81, or 1.36 higher than Manila and only 1.48 lower than Kuala Lumpur. It had an abnormal maximum in September, 1911, of 20.77, or higher than either of the so-called tropical places and a minimum of 3.48. However, the average of days above the average mean is 15.82 as against 16.52 for Kuala Lumpur. No months in Honolulu are as low as the lowest in Manila (September, December; 10.94 and 10.03 respectively). Therefore, Honolulu (21° 18′ north) has, as regards the photocatalytic action of the sun's rays, a climate much like that of Manila (14° 36′ north) and Kuala Lumpur (3° 10′ north), and the extraordinarily high days observed at that place indicate that at times the atmosphere on Hawaii is so free from disturbances, strata of varying density, or haze, as to allow even a greater proportion of the rays having photocatalytic action to reach the surface of the earth, than is the case in the more southern places. No one will venture to state that the sunlight is more oppressive in Honolulu than in the Philippines; indeed, the general temperature is lower, the average temperature at the time of the observation was 21°.1 to 22°.6, where ours in Manila was 30° to 35°, so that, if the slight temperature coefficient for the differences were to be taken into consideration, Honolulu [16] would result even higher. The difference between these three places under discussion is

[16] The figures from Honolulu are not final as the flask used was a 200-cubic-centimeter Jena glass Erlenmeyer and has not yet reached us for standardization. This will probably make the rate high, but, on the other hand, our standard is a quartz flask, which would offset the increase due to greater surface in the Honolulu flask, so that probably but little correction will be necessary in the end.

so slight that we can say that practically the photocatalytic action in all is the same.

Unfortunately, only a few data have reached us from Tucson, Arizona (32° 12′ north) (Table IV), and these for the month of October. They show a maximum of 13.4, or 4.4 less than that of Manila, and a minimum on one day of 7.7 or 2.5 greater than the average at this place. The temperature during the observation averaged about as it does here (28°.7), and higher than at Honolulu. Doubtless, when a longer series of observations is at hand from this interesting point, we will discover many days in Tucson where the maximum is as high as, or higher than, it is here, and an average about the same.

The data from Washington (Tables V and VI) need a little more careful analysis, as the methods followed were not always identical with the ones adopted by us as a standard, the hours of insolation were not always the same and recalculations need to be made in that respect. Nevertheless, so far as they are comparable, the results show that Washington, which has a winter climate, presumably more atmospheric disturbances, and many cloudy days and possibly but few absolutely clear days, can show at times as much effect as the four places discussed and an astonishingly high average of 11.80. One day in September gave an hourly decomposition, between 8.45 and 12.15 in the morning, of 19.14 per cent, and making allowance for the greater concentration of uranyl acetate used by Doctor Bacon, it would still be close to 16.00; and, in February, between the hours of 9 and 1, a decomposition, which, making allowance for the temperature coefficient and for the concentration of uranyl acetate, would certainly in Washington show but little difference between summer months (July, August, September), 11.0, and the winter months (December, January, February), 10.0. Making allowance for the greater concentration of uranyl acetate used by Bacon, the totals in Washington are lower by about 33 per cent than in Manila, excepting the one month, November, in Manila with an average of 9.97.

The results in Khartoum, Sudan (Table VII), are extremely interesting and, perhaps, the most instructive of the series. Khartoum is close to the desert and in about the same latitude as Manila. We find here, in observations extending through the months of September, October, and November, an average of 17.6 as measured by a standard quartz flask, or as much as 5.15 higher than Manila and 2.3 higher than Kuala Lumpur,

but this average is so high because of the remarkably uniform character of the insolation, the minimum being 14.7 as against 9.0 for Kuala Lumpur and 1.15 for Manila. The maximum observed day at Khartoum was 20.8, which is higher than any observation at Manila by 3.0, and 2.7 more than the highest observed at Kuala Lumpur, only two other observed days approaching this, one of 20.7 at Honolulu and the other 20.6 at Baguio in the Philippines, at an altitude of 1,432 meters. In Khartoum, out of sixty-six days of observation, no less than fifty-two gave decompositions between 16.7 and 17.9 and eleven between 17.9 and 18.6. In Khartoum, therefore, we have a remarkably uniform, high insolation so far as the portion of the spectrum under consideration is concerned; but, nevertheless, the days of maximum illumination do not materially differ from those in the other localities, so that the absolute intensity of the ultra-violet illumination which *may* reach the earth on perfectly clear days does not materially differ, the distinction being meteorological. If we consider this uniformly high rate and its causes, it is evident that the reverse can also be true and it would be possible to have so-called tropical climates where cloud interference and other causes would bring the average illumination below that in temperate zones. The temperatures of observation at Khartoum were somewhat higher than at Manila and Kuala Lumpur, but we observe that days of maximum temperature are not necessarily days of maximum photocatalytic decomposition.

Another interesting comparison is furnished by Bruner and Kozak[17] working in Krakau (53° 40′ north) on bright, sunshiny days in the spring and summer, the solutions in test tubes being exposed between the hours of 10 and 2. The background is not stated, but as they worked before an open window it is to be presumed that reflections did not play as important a part as with flasks placed on white paper, although the buildings had to be considered. As the work was done in test tubes, we can not conclusively compare results, the variation owing to the shape of container might be considerable in amount, but still, these authors, with a solution corresponding to our standard, obtained a decomposition of 15 as the maximum in their observations, so that it is apparent that even in this latitude days occur with a photocatalytic reaction sufficiently high to be comparable with those in the Tropics.

[17] *Ibid.*, 35.

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[17] *Ibid.*, 35.

In order to compare a climate at higher altitude and but little north of Manila with that of the latter city itself, a series of observations was made at Baguio (Table VIII) (altitude 1,432 meters). The temperatures of the nights and in the shade at Baguio are so low that it is an ideal resort for recuperation from the lowland climate, yet the photocatalytic action is much the same, however, with this difference: The maximum at Baguio is higher than in the lowlands (20.6 as against 17.8), being in this respect like Honolulu (20.7). The average is 14.2, or 1.75 more than in Manila and 1.09 less than Kuala Lumpur and 0.39 more than Honolulu. The ascent of 1,432 meters has produced the same effect on the photocatalysis as a transfer to Honolulu. The black-bulb readings are practically the same. At Baguio, therefore, as we would expect, we encounter a climate in which the rays undergoing investigation are somewhat more intense than in the lowland. The average temperature in the sun during the observations was 7° to 8° lower than in Manila.

Manila and Baguio, at present, are the only places where the black-bulb thermometer readings are available simultaneously with the photocatalytic measurements, and a study of individual days demonstrates that the two figures, namely, black-bulb readings and percentage of oxalic acid decomposed are not by any means functions of each other; indeed, within reasonable limits they seem to be independent. Of course, it is understood that a certain relationship exists, because, naturally on clear, bright days both black-bulb and photocatalytic readings will be high, and both the reverse on cloudy ones. As an example of these variations, I can cite a few figures taken from daily observations:

Comparison between photocatalytic and black-bulb readings in Manila.

From 9 to 12 a. m.	Weather.	Photocatalysis.	Black bulb (mean of 3 observations)
1910.			°C.
April 28	Clear	15.4	52.0
May 7	do	17.7	52.5
May 16	Slightly cloudy	13.4	54.0
May 18	Clear	16.4	52.0
June 9	do	14.7	54.5
July 5	Slightly cloudy	16.6	56.3

Comparisons of this kind can be extended almost indefinitely, but those given suffice to show that, in the same place and on apparently equally clear days, the relative proportions of the

rays in the various portions of the sun's spectrum may vary considerably.

So much, for the present, for the effect of the more refrangible rays of the sun's spectrum lying in the region of the blue to violet and beyond in the ultra-violet. To them, the greater part of the literature has attributed, in largest measure, the supposed untoward effects of the tropical sun, and to them have been attributed even grave morphologic changes sufficient to bring about permanent differences in races of the human family. So far as the work has gone it seems to develop that, if the so-called "actinic" rays in Manila are particularly objectionable, they are the same in Honolulu and for a certain time of the year even in Washington. However, the more we consider the ultra-violet rays of the sun's spectrum, taking cognizance of the fact that nowhere, whether in northern climates or in tropical ones, do they extend beyond 291 $\mu\mu$, understanding what a large proportion, if not all, of the direct rays are subjected to molecular scattering, reflection, and dispersion by the upper layers of the atmosphere, and noting the slight differences between the lowlands at Manila and highlands at Baguio, we are forced to the conclusion that, on clear days, when the sun is at the same angle, they are everywhere much alike in intensity. Indeed, it appears as if the greater part of these rays which reach the earth are diffused and not direct.

These considerations bring us to the much larger remainder of the spectrum which extends upward from the point mentioned into the red and infra-red and which would include the heat rays. That these are a most important factor is, of course, self-evident, and so we, in considering the subject, have not overlooked this fact, but means of direct measurement as in the case of a photocatalytic reaction are lacking. The black-bulb thermometer is variable and unsatisfactory. Better comparative data could be obtained if a series of readings of the total solar radiation per square centimeter of surface, normal to the ray of incidence, were available with the Angström pyrheliometer.

Such data as are available have been gathered by Dr. Herbert H. Kimball of the Mount Weather Observatory [18] in a summary which gives the most important figures for the present discussion. Comparisons are made of the annual maximum intensity of solar radiation at various points as follows:

[18] *Bull U. S. Mt. Weath. Obs.* (1910), 3, 100.

Station and latitude.	Intensity.
Cape Horn, 55° 31′ S.	1.47
Washington, 38° 54′ N.	1.44
Montpelier, 43° 36′ N.	1.60
Modena, 44° 39′ N.	1.37
Kief, 50° 24′ N.	1.39
Warsaw, 52° 13′ N.	1.35
Hald, 56° 25′ N.	1.32
Katherinenburg, 56° 50′ N.	1.58
Pavlovsk, 59° 41′ N.	1.48
Upsala, 59° 51′ N.	1.35
St. Petersburg, 59° 56′ N.	1.47
Treurenburg, 79° 55′ N.	1.29

These variations are not great, and such as appear, are attributed by Kimball to instrumental rather than to atmospheric conditions. Angström [19] publishes some results from Teneriffe (20° 30′ north) in which he compares Guimar (360 meters altitude) with Alta Vista (3,852 meters altitude) and obtains 1.38 at noon for Guimar and 1.618 for Alta Vista, the latter higher figure is to be expected owing to the altitude. The maximum observed by Dr. Rudolph Schneider at Vienna (48° 13′ north) [20] was 1.524 in February, and figures ranging from 1.00 to 1.455 are quite frequent; indeed, the observations for the time close to the noon hour in Vienna, although averaging somewhat lower, bear a remarkable resemblance to those in Washington, when we consider that Kimball worked only on clear days and Schneider made observations on days of partial cloud and even of fog. Mr. Harvey N. Davis, working at Providence, Rhode Island, in ten months observed a maximum of 1.328 in March, and in general his figures also bear a striking resemblance to those obtained in Vienna. Kimball, in discussing the annual march of radiation as compiled by him, states that "a rather surprising uniformity throughout the year (is shown) in the maximum intensity of radiation, the December minimum being only 8 per cent less than the April maximum." The departures by months from the average quinquennial mean show that there is a considerable variation by years, amounting to a minus quantity of as much as 18 per cent on the average for the year 1903. This diminution was widespread and such low times are periodic; the same is probably true of high periods, so that the absolute amount of insolation on the earth's surface may vary

[19] *Astrophys. Journ.* (1899), 9, 342.
[20] *Jahrb. d. k. k. Zentralanstalt f. Meteorol. u. Geodyn.* (1906), n f. 43, 12.

from year to year,[21] but such variations are not frequent enough or of great enough intensity to alter the picture as a whole.

Although the maximum radiation at the various points mentioned is very similar in all, yet if we take the annual totals, we find differences for such points as have been compared. Kimball [22] has calculated the average monthly totals for Washington and Warsaw for normal surfaces, and from them we can obtain the yearly totals, which for Washington are 254,026 and for Warsaw 216,200, so that Warsaw actually has 85 per cent of the radiation received at Washington, although it is 14° farther north.[23] Unfortunately, pyrheliometer readings for places in the Tropics are not at hand. We have ordered an Angström pyrheliometer nearly a year ago, but the instrument has not yet arrived. When it does, we will begin readings in Manila and thus obtain comparative data. Enough has been shown already to demonstrate that meteorological phenomena, percentage of possible sunshine, and varying atmospheric transmissibility have more influence than variation in the actual solar insolation on perfectly clear days, and it is evident that such factors can just as readily be introduced in the Tropics as in other regions of the earth.

Because data with the Angström pyrheliometer in the Tropics are lacking, we attempted to solve the problem, for the present, by having recourse to animal experiments.

In considering this second phase of the question, a few fundamental facts must be borne in mind. The air surrounding the earth absorbs the rays of the sun in a certain proportion, and another part is reduced by reflection, molecular scattering, and dispersion; this takes place in a greater proportion with the more refrangible than with the less refrangible rays, so that the light reaching the earth contains relatively a greater amount of the rays of the upper range of the visible spectrum and infrared than are in the sunlight before it strikes the atmospheric layer; indeed, all ultra-violet rays up to 291 $\mu\mu$ disappear. On the other hand, the dark heat vibrations of great wave length, radiating from the earth, are absorbed in great measure by the atmosphere. The coefficient of absorption of the air increases with increasing density, but it never reaches that of a solid or

[21] Kimball, *Ibid.*, 114, 115.

[22] *Ibid.*, 103.

[23] These computations for Warsaw cover the period from July 1, 1904, to December 31, 1906; the period for Washington from June, 1905, to March 21, 1910.

liquid substance, such as the soil and water which form the surface of the earth. The power of absorption of the air is influenced by such factors as humidity, actual nuclei or droplets, clouds, and other causes, just as it is by density; but moisture-laden air relatively does not absorb as great a proportion of the rays of lower refrangibility as it does of higher. It is for this reason that air temperatures at higher altitudes are lower than in the lowlands, although the effects on solid objects, such as the black-bulb thermometer, may be greater. This may be shown by a comparison of some black-bulb thermometer readings in different parts of the world, which I have gathered for other purposes. At Davos, Switzerland (altitude 1,559 meters), the average of maximum black-bulb readings for three years was 53°.8, with a highest absolute maximum of 67° in 1910. Compare this with Manila, where the maximum for one year (1910–1911) was 56°; or with Helwan, Egypt, where the highest observed was 70°.8 during a period of three years; or with Alexandria, Egypt, with a maximum of 57° during the same period. Of course, there are places on the edge of the desert, where the atmosphere is exceptionally clear and where reflected light is present in great proportion, that exceed these figures, so, for example, Cairo, in May and August, 1909, shows a maximum of 79°.5; and Aswan Reservoir, in June, 1910, of 81°. However, in contradistinction to these desert places, we have another remarkably high black-bulb reading at high altitude, in Leh (Thibet), (altitude 3,517 meters) of 101°.7 with a shade temperature of 23°.9. Of course, these figures refer to maxima only, and do not take into consideration averages, or the shade temperatures, which may be high or low, but it is evident that the occurrence of days of extreme insolation is not so much a matter of latitude as of situation, and it is evident that even in the Tropics we might come to averages decidedly lower than in certain more northern, temperate climates. It is obvious that in any one of the places mentioned, a living body might encounter days in which it would be heated by solar radiation to a much greater extent than in the Tropics, and the only question would be whether the possibility of cooling, such as is brought about by low air temperature, low humidity, wind, or other means would compensate to avoid the effects of such insolation.

A body exposed to the sun absorbs a portion of the rays and reflects a portion of them, the most perfect absorption being that of as nearly ideally black a substance as is possible. The body would go on storing the energy so conveyed to it indefinitely,

were it not to lose it by radiation or convection (conduction), and the rate of this loss increases in proportion to the energy added to the body by radiation, until an equilibrium is reached. Black bodies, while absorbing the radiations readily, also radiate readily, so that it may come about that a black surface, exposed to the sun, may become little, if any, hotter than one of lighter color under similar circumstances.

It is a well-known fact that the ultra-violet rays are promptly fatal to almost all the lower organisms, such as bacteria, amœbæ, and protozoa; the heat effect on them being much less, and only apparent in so far as above certain temperatures they can not live. As we ascend high enough in the orders of animals, devices for regulating the losses of heat begin to appear, until, in birds and mammals, they are so well developed that but little variation in blood temperature is observable under the most diverse conditions of life, and hence a study of the effects of the lower rays of the sun's spectrum on such organisms, under normal and abnormal conditions, is most promising.

Such a study was undertaken by Dr. Hans Aron of the department of physiology of the University of the Philippines in conjunction with our other sunlight work, and his first results have recently appeared.[24]

The first problem was to construct apparatus for thermometric work which could easily be handled so as to give the subdermal, rectal, and skin temperatures quickly and accurately within 0°.1. This was finally accomplished by a series of specially prepared thermocouples, temperatures being read by a tangent galvanometer.[25]

Perhaps the most instructive and interesting results were obtained with monkeys, animals which naturally are at home in the Tropics and which, we should suppose, would best be able to withstand the effects of sunlight. The system of sweat glands in monkeys is not so highly organized as in man [26] and

[24] *This Journal, Sec. B* (1911), 6, 101.

[25] *Ibid.*, 117.

[26] Aron, *Ibid.*, 110, makes the statement that monkeys have no sweat glands. During the time at his disposal, as he was going on long leave, Aron did not investigate this question completely. Doctor Shaklee of the department of pharmacology, University of the Philippines, states that monkeys do have sweat glands. See also Blaschko, *Arch. f. mikros. Anat.* (1887), 30; Wimpfelheimer, *Anat. Hefte* (1907), 34, 492. Krause, Beiträge z. Kenntniss der Haut d. Affen; Inaug. Dissertat., Berlin (1888) is not available. Sweat glands have been found by Mr. Clark of the department of anatomy, University of the Philippines, in the forehead, hands, feet, axillæ, and abdomina of our monkeys.

their physical heat regulation is to a much greater extent brought about by water evaporated from the lungs and mouth through increased respiration. The normal subcutaneous temperature of the animals, in the shade, varies from 36°.6 to 38°; the rectal from 37°.9 to 39°.4. The subcutaneous temperature, therefore, is somewhat below the rectal. However, as soon as the animal is placed in the sun, the subcutaneous temperature rises above the rectal and remains so to the end of the experiment, so that the inside of the body now receives heat from the periphery. The animals exposed to the full sun,[27] without protection or artificial means of lowering the temperature, die in from one hour to one hour and fifty minutes; the exposures being either in the morning between 10 and 11, or in some cases in the afternoon between 2 and 4, in the months of November and January. Both the skin and rectal temperatures steadily rise during these exposures, the maxima before death being 43°.5 and 42°.7 to 46°.3 and 44°.8 respectively.

Entirely different results are obtained if the animals are shaded, even by a small area of shade such as an umbrella or a board, all other conditions being similar, so that the direct rays are excluded, the diffuse rays, excepting those cut off by the shade, still being available. Under these circumstances the skin and rectal temperatures never exceed 40° and the animals remain healthy. Similar results are obtained if the animals are exposed to full insolation, but care is taken to conduct away the excessive heat increment by means of a brisk current of air from a fan. Under these circumstances the subcutaneous and rectal temperatures remain the same as when the animal is shaded, never rising above 40°.6, and the monkey remains perfectly well. In this last form of experiment the monkey is exposed to all the rays of the sun, including those of lesser refrangibility, heat waves alone being conducted away. If untoward effects are to be attributed to the absorption of the ultra-violet rays, then surely the animal is in the same condition to absorb the latter as he is when no blast of air is present, and their effect should be apparent. On absorption, a large proportion of these rays is presumably converted to heat and conducted away as such, so that it can be assumed that the effects which we observe on exposing these animals to the sun is one of heat, and these conclusions are borne out at autopsy where post-mortem exam-

[27] The proportion of the body exposed to the rays in the full sunlight, even toward noon, is the lesser part of the whole, as more than one-half of the body is in its own shade.

inations give protocols clearly pointing to heat stroke. Monkeys enclosed in tight boxes, with only the head exposed, and placed in the full sun, suffer no inconvenience, although the hair temperature on the scalp may reach 47°. The effects, therefore, are not due to penetration of the sun's rays to the brain. Of course, it must be understood that the monkey's skin is protected by fur and is not sensitive to the irritating effects of the ultra-violet rays, such as would be the skin of a Caucasian[28] who, as we all know, if exposed to the sun, would be sunburned, whether in a strong blast of air or not. This latter effect is due to the ultra-violet portion of the spectrum, and as the latter rays have but little power of penetration the skin can in time amply protect itself by pigmentation. Even though pigmented, as is the monkey's skin and hence not subject to sunburn, the heat effect would still remain and bring about the results of excessive heat exposure in exactly the same manner as in the case of the monkeys. The ultra violet rays are easily guarded against, the heat rays not.

Experiments on man exposed to the sun are equally interesting. In man we have a subject with highly developed sweat glands, so that the means of heat regulation by evaporation are much more complete than in dogs, rabbits, or monkeys.

Skin temperatures of men in this climate in the shade under normal conditions vary, as measured by the apparatus constructed in Manila, within the extreme limits of 31° to 34°, being higher over the muscular and fatty parts of the body than over bony structures lying close to the surface. These variations must be considered and therefore measurements on the changes of temperature, when exposures are made, must be taken at various points of the body.

After ten to fifteen minutes' exposure to the sun, the skin temperature of an American subject, on the sunny side, rose to 35°.8, 35°.2, and 41°.8 on the arm, cheek, and chest in the order named, whereas on the shaded side these temperatures were 31°.5 and 31°.9 on the first two; the hair temperature rose to 46°. The corresponding temperatures in a Filipino, after thirty minutes, were 36°.9, 35°.4, and 39°.8, the shade temperatures being 32°.5, 31°.9, and 32°.5 the differences being, in regard to the brown skin, +1°.1, +0°.2, and −1°. A comparative measurement of an American and Filipino, side

[28] Aron exposed a shaved monkey. It died within one hour, with the same autopsy protocol as others. Its temperature rose more rapidly than that of the others, reaching 45°.5 and 44°.4.

by side, on the same day, after fifteen minutes' exposure was as follows: American, 35°.6 and 34°.0 on the arm and cheek; Filipino, 34°.8 and 33°.9; the difference being −0°.8 and −0°.1 in favor of the Filipino. In another series of experiments, there were compared a Spanish-Eurasian and a Filipino with dark brown skin. After one-half hour in the sun, the records were as follows: Spanish, 37°.1, 36°.5, and 35°.0 for the arm, face, and the back of the neck; whereas for the Filipino they were 36°.3, 36°.3, and 34°.6, differences of −0°.8, −0°.2, and −0°.4 in favor of the latter; and in a second series, after ten minutes, 36°.2, 35°.2, and 35°.4 as against 36°.2, 34°.1, and 34°.8; differences of 0°.0, −1°.1, and −0°.6. These differences are but slight as between the white and dark skin, the majority of observations being somewhat in favor of a lower skin temperature for the Filipino, but after fifteen minutes both the American and Spanish-Eurasian were sweating slightly, whereas this was not apparent with the Filipinos. Another series of results was obtained after longer exposures, when all of the subjects were sweating freely and only slight differences were observed, thus the Spanish-Eurasian after forty-five minutes in the sun, having performed muscular work, showed temperatures of 33°.2, 33°.0, and 33°.2 as against 33°.4, 32°.6, and 32°.8, differences of −0°.2, −0°.4, and −0°.4. At rest, lying on a cot, after one hour's exposure, the skin temperatures were 34°.6, 35°.2, and 35°.0 as against 34°.8, 34°.8, and 34°.4; differences of +0°.2, −0°.4, and −0°.6. It will be seen that the skin temperatures, at rest, do not fall as rapidly as when the subject is doing muscular exercise, but yet, after one hour, they are from 0° to 1°.6 lower than the earlier maximum, except in one observation, when the rise was 0°.7. The fifty minutes of exposure, therefore, have caused no practical rise over the temperatures after the first ten minutes, and indeed a lowering in all but one instance, the excessive heat received by radiation being taken care of by the usual means and by evaporation through perspiration, whereas in the case of the monkeys there was a steady rise up to the lethal point. In a final series of experiments, an American and a Filipino were exposed side by side for thirty minutes. In this case the final temperatures were as follows: American, 36°.9, 36°.3, and 36°.5 as against 36°.1, 35°.4, and 35°.4 for the Filipino, differences of −0°.8, −0°.9, and −1°.1 in favor of the dark-skinned man. Therefore, out of 14 observations, 12 showed a lower skin temperature for the Malay race, so that the series results slightly in favor

of the darker skin, the highest of all observations being 37°.4 in an American, on the cheek, after exposure for twenty-five minutes.

These measurements, while showing conclusively that the adaptable mechanism for heat regulation possessed by human beings is sufficient to lower the temperature and protect the individual from such fatal effects as are observed in monkeys, still did not appear sufficiently conclusive as regards the differences between the white and dark skins. Therefore, both for the purpose of comparing the effects of insolation at high altitudes with those at sea-level as well as for a further study of the possible differences between the two colors, the experiments of Aron were repeated in Baguio by H. D. Gibbs of the laboratory of organic chemistry of the Bureau of Science. The skin temperature in Baguio rose to higher points than those observed by Aron in Manila.

This may in part be accounted for by the technique employed, for Aron warmed the thermometric junction in the palm of the hand and then placed it on the part of the skin to be measured, whereas Gibbs commenced measurements a short distance from the desired spot and, as soon as the maximum deviation of the galvanometer was reached, moved the thermocouple nearer the place, and when the instrument was again at rest, placed it in the final position. However, after taking the differences in technique into consideration, the absolute values recorded for the upper altitude are still higher than those for the lower.

Comparison between an American and two dark-skinned Igorots, A and B, taken over the level of the third dorsal vertebra, the fifth dorsal vertebra, and over the upper angle of the scapula, in the order named, were as follows: The average of shade temperatures of all subjects being 30°.06, 32°.4, and 33°.52, but it must be recorded that a slight breeze affected the American's temperature.* After twenty-seven minutes the American reached maxima of 37°.65, 37 .15, and 37°.95; Igorot A, after thirty-six minutes, measured 38 .05, 38°.35, and 37°.9; whereas Igorot B, after thirty-three minutes, recorded 37°.4, 37 .9, and 36 .8, or temperatures averaging +0°.73 against the dark skin of A and 0 .05 in favor of B. The thigh of A, which was steadily exposed to the sun, showed the remarkable skin temperature of 52 .7.°

* Wind screens were used to protect the subjects from the cooling effects of the breeze, but occasional eddies would reach the men in spite of all precautions

In the first ten to fifteen minutes the temperature of the white skin rose more rapidly than the dark; namely, an average of 6°.25 as against 2°.60, but then the white skin began at a lower shade temperature; and if the difference is taken into account, the white skin rose 2°.79 as against 2°.60, so that there is but little difference in this respect. In the final temperatures, one Igorot reached 0°.73 more than the American, while the other was practically the same.

In a second series of measurements, a Canadian, a Filipino, and an American Negro were compared. The shade temperature averaged 33°.65, 33°.97, and 34°.15, or higher by 0°.63 to 3°.59 than in the previous experiment. After thirty-one minutes the Canadian reached 38°.55, 37°.40, and 38°.85; the Tagalog and Negro, after twenty-nine minutes, reached 38°.75, 38°.80, and 38°.78 and 39°.32, 38°.85, and 39°.15 respectively. Therefore, the differences were +0°.20, +1°.40, and −0°.07 against the Tagalog and +0°.77, +1°.45, and 0°.30 against the Negro. In the first seven minutes the Canadian recorded a rise of 4°.3, 3°.2, and 4°.35; the Tagalog, in eight minutes, 5°.55, 5°.40, and 5°.25; and the Negro, 3°.90, 3°.95, and 4°.50, so that the temperature of the Tagalog rose decidedly more rapidly than that of either of the others, but the Negro had an initial shade temperature higher than those of the Canadian and Tagalog by somewhat more than 1°.0. The final temperatures, therefore, are decidedly against the Negro, slightly so against the Tagalog, and in favor of the Canadian as against the other two. Taking these experiments into consideration and comparing them with the indices we use in Manila, it may be said that, as regards rise in temperature on exposure to the sun, the white and brown skins are about equal, with a slight factor in favor of the white, but that in the case of the very dark-skinned Negro, the temperature on exposure reaches a decidedly higher point than it does with either of the others.

One fact very strikingly appears from these measurements, namely, that the skin temperatures of all the subjects reach higher points in the sunlight at the high altitude of Benguet than they do in Manila, despite the lower shade temperature at the former location. However, the measurements show that perspiration begins at an earlier period in the lowlands.

In explanation of the above results, it may be taken for granted that the dark skin of the Negro will absorb heat more readily than the light ones of the American or Canadian, but

then, it will also radiate more readily, so that heat rapidly taken up on the sunny side will also rapidly be lost on the shaded one and it is the balance between the two which determines the ultimate degree of rise in temperature. This balance evidently results against the Negro. On the other hand, with the white skin we have the phenomenon of sunburn, with its resultant irritation of the nerve-endings and hyperæmia of the peripheral tissues, and this would cause a rise which, apparently, just about offsets the rise in the brown skin due to the pigmentation.

The decidedly higher skin temperature of the Negro made it of importance to investigate the behavior of animals of such decided differences in color that the contrasts would show with greater certainty. For this purpose 6 rabbits: 2 pure white, 2 gray, and 2 black, were used. These were placed in the sun, side by side, with only a few centimeters between, the subcutaneous temperature being taken through a small slit in the lower dorsal region. The first 3 animals remained in the sun for thirty-six minutes, from 9.10 to 9.46 in the morning, at which time they were returned to the shade. The white and gray rabbits soon recovered from the exposure, but the black one died at 12.30 in the afternoon.

The subcutaneous temperatures rose from 38°.6 for the white and gray and 41°.8 for the black to a final height of 41°.0, 42°.8, and 44°.2 for white, gray, and black in the order named.

In the second series the black and gray animals were strong and healthy specimens, whereas the white was much weaker.

The subcutaneous temperatures at the beginning were 38°.0, 37°.85, and 37°.7; the exposures were for one hour and thirty minutes, from 9.02 to 10.32 in the morning. The black rabbit reached a maximum of 47°.8 in thirty-one minutes and then died; the gray rabbit, a final temperature of 44°.9 in one hour and twenty-six minutes, when it died; the white rabbit a final temperature of 45°.7, and when put in the shade, it recovered although much exhausted.[30]

These experiments appear conclusive. None of the animals suffer from sunburn as does the white man, and it is evident that the darker the coat, the greater the heat absorption and the more apparent do the effects of insolation become. It appears

[30] Monkeys exposed to the sun at Baguio developed higher subcutaneous temperatures than in Manila; a maximum of 54° being reached in one case before death, and in another 48°.3 before death. Rectal temperatures were not taken.

evident when these results are compared with those observed for human beings that, all other things being equal, the Negro will suffer more from the heat effects than the lighter-skinned races.

Chamberlain,[31] in a series of observations in which he carefully compared the relative resistance to the Philippine climate of blond and brunette types of soldiers, concludes that the evidence is conflicting and that from a consideration of all the facts the blonds are quite as well able as the brunettes to withstand the Philippine climate. The effect of the rays of greater refrangibility in the violet and ultra-violet portions of the spectrum are not the important factors, except in so far as they cause sunburn and subsequent excessive pigmentation, but protection from these rays is so easily accomplished and has been accomplished so long as man has worn clothes, that skin-color can not be an important factor in determining adaptability to climate; that question is a morphologic one which takes into consideration many more factors than skin-color alone. A white cotton shirt and white trousers are sufficient to protect against sunburn, and hence against the ultra-violet rays.

Phalen[32] compared 500 troops in the Philippines, dressed in orange-red underclothing with 500 dressed in white. The experiments show that the test underclothing added materially to the burden of heat upon the system and that the white underclothes of practically the same weight were superior in this respect. In fact the lighter and whiter the clothing, the better is it adapted to protection against the sunlight; indeed, in the Tropics, were it possible, the ideal protection simply would be an umbrella. The lowering of temperature in man is brought about by evaporation of perspiration, and the better the facilities offered for this purpose, the better off will the individual be.

Consequently, relative humidity plays a most important part in the study of the influence of the sunlight. The higher the relative humidity, other things being equal, the less readily will evaporation take place and the less complete will be the result in lowering the temperature. As the lowering is brought about by the evaporation of sweat, it necessarily follows that those races with the best developed sweat glands will have an

[31] *This Journal, Sec. B* (1911), 6, 427.
[32] *This Journal, Sec. B* (1910), 5, 525.

advantage. The greater the surface for evaporation, the greater will be its effect. For this reason it seemed advisable to investigate the relative number of sweat glands developed in the white and Malay, and Mr. Elbert Clark of the department of anatomy of the University of the Philippines has pursued this subject. After many measurements on American soldiers, Philippine scouts, and persons of both colors in civil life, he has come to the conclusion that the Malay possesses from 12 to 15 per cent more sweat glands than the white. Measurements on Negroes are not yet complete enough to warrant a final statement, but the results, so far, show that the race has perhaps an excess of 7 per cent. The few counts which have been made on Negritos give 26.82 per cent excess for adults and 67.54 for youths.[25] Neither can anything be said as to the relative capacity of the individual glands in the two races.

In this respect, then, the Malay possesses a decided advantage over the white man which the latter can only offset by seeking greater shade, but, to judge from the data which have been given, ample protection at all times can be given to all races by sufficient shade, as owing to that protection the temperature does not rise, and indeed is somewhat lower, apparently, in the white. Given ample shade, and any race is adapted to resist the sun alone of tropical climates; the white man should be better able to do so than the colored. It would seem to me as if the dark skin of the Negro was not a result of excessive insolation, for it is certain that in a state of nature the Negro would seek the shade, just as monkeys do, intuitively, and in the earliest times he probably was exclusively a forest dweller. The color of his skin would, therefore, more probably be protective just as protective coloring is developed in animals other than man.

One other factor must be considered in discussing the influence of latitude upon the total heat effect throughout the year, and this factor would not in general appear as such by any of the means of measurement mentioned in the previous part of this paper, namely, the absorption of heat by the earth's surface and its radiation therefrom. This factor will naturally vary with different regions according to the color of the surface exposed,

[25] Measurements on Negroes when continued in a longer series will probably result in higher figures. The endeavor will be made to secure more Negritos.

being least in green surfaces of vegetation and greatest in rocks or red, clay soil such as is common in India under the name of laterite. The actual number of hours of insolation per year on the earth's surface, were the sky always clear, is greatest at the equator and diminishes toward the poles, the ratio between 0° and 45° being 1.83 to 1.34, although in the longer days in the temperate zone the sunshine reaching the earth when the sun is near sunrise or sunset is only a small proportion of that at midday. As a result we have in the Tropics the added factor of greater radiation from the earth's surface to augment the direct influence of the sun, so that, as it has been shown above that the influence of heat is the chief one to consider, this increment due to radiation from the earth would be of decided influence in the Tropics. In middle latitudes this factor has been determined as about 0.1 of the solar insolation at midday, but it acts during the entire twenty-four hours, whereas the sun rises and sets.[34] In northern climates the hours of insolation during the short days are so few and the hours of radiation so many during the night that the surface of the earth actually steadily cools at certain times of the year, making one of the factors which causes a winter season.

Probably, untoward effects attributed to the tropical sun, if any, are caused by the evenness of the climate rather than by the differences of insolation at any one time; the absence of severe contrasts, such as are given by the winters and the monotony having their effect. However, Chamberlain [35] investigated the systolic blood pressure and pulse rate in 6,847 readings in 1,489 individuals of varying lengths of residence in the Philippines and found that:

"Reduced to the basis of a 12.5-centimeter armlet * * * the average blood pressure for healthy white men in the Philippines (is) 115 millimeters for those between 15 and 30 years of age and 118 millimeters for those from 30 to 40 years old. These figures are little if any below those to be expected in a temperate climate when a 12.5-centimeter cuff is employed. * * * There was no progressive tendency for the pressure to increase or to decrease with continued tropical residence up to a little over three years, beyond which point our observations do not extend." This author also found that "we may * * * conclude that the mean blood pressure for

[34] Hann, Handbuch der Klimatologie (1910), 2, 28, calls attention to measurements in Chinchochro, Laoango coast, near the equator. The regular measurements of the surface of the earth exposed to the sun gave temperatures generally over 75°, often 80°, and one time nearly 85° C.

[35] *This Journal, Sec. B* (1911), 6, 437.

Filipinos during the period of 15 to 40 years of age (average about 25 years) is 115 to 116 millimeters and that it does not differ from the pressure at the same ages for Americans residing in the Philippines. For neither race is it very materially below the figure to be expected for white men residing in temperate climates."

Mr. H. D. Gibbs has endeavored in the laboratory of organic chemistry of the Bureau of Science to determine what changes are brought about in the animal economy by exposure to severe sunlight, and has obtained indications in rabbits of the formation of methæmoglobin, but the work is not sufficiently advanced to be definite. A report on these results will therefore be postponed.

From all of our observations it would seem legitimate to draw the conclusion that a climate such as we have in the Philippines, where we are surrounded by the sea which modifies the extremes of temperature and where we have such a large proportion of cloud, is not by any means deleterious to the white man if he takes ordinary precautions which are not as elaborate as those he would take in a northern climate to keep out the cold. The differences in maximum insolation as compared with temperate regions are not great, if any, and many days occur in which the effect of the sunlight is greatly modified. The individual needs only to seek the shade to avoid any deleterious results from even the greatest insolation. If individuals must be exposed to the sun, as is the case with troops on the march, they can be given adequate protection by light, preferably white, clothing and helmets, but it must be remembered, as shown above, that perspiration is a great factor in keeping the man normal under these conditions and that, during exercise in hot weather much water is lost during the day. Many of the untoward effects attributed to the sun are probably due to the rapid loss of water from the system and could be avoided if the individual were in a position to drink enough to preserve the equilibrium. Two canteenfuls per man are certainly not sufficient. The temptation to drink available water along the road, also, may become irresistible, and sickness caused by infection from such a source may be attributed to the sun as a predisposing factor. Even in places like Khartoum, where the average effect of insolation is much higher than in Manila, the results can be avoided just as they can be here, and it is only in the places where the radiation from the earth at night is so great that no relief is experienced from excessive heat, that the climate may become such as to preclude the possibility of persons unaccustomed to such conditions living in health. In the Philippines

the nights are rarely too hot for comfort and they may even be quite cool.

Before concluding, I wish to call attention to another phenomenon to which I referred two years ago in a previous paper on this subject and which, at the time, I said merited further investigation. Bacon [36] observed that the fall of the aluminium leaves in a fontanoscope, according to Engler and Sieveking, was much accelerated when the apparatus was placed in the sunlight, as compared with the dark. At that time this result was attributed to the ionization of the air by the sunlight of Manila. Since that time, we have modified the apparatus by carefully enclosing it in a glass jar which could thoroughly be dried and which avoided outside influences. Almost 2,000 readings were made both in Manila and Baguio with currents varying from 300 to 1,000 liters per hour. The data are too voluminous to quote here, but will be published by Mr. H. D. Gibbs of the laboratory of organic chemistry, Bureau of Science, at a later date. The rate varies from day to day, but with the modified apparatus the highest fall was far below those obtained by Bacon, and a careful analysis shows that the rate during sunlight does not materially differ from that during cloudy weather. Bacon's results can therefore be attributed to outside disturbing factors, as the apparatus during Mr. Gibbs's measurements was removed from outside influences. This work is being continued so as to include the total ionization of the air in Manila and will also give the data in regard to the ionization due to radio-activity, the measurements for which are being made by Dr. J. R. Wright of the department of physics of the University of the Philippines. Simpson and Wright [37] in a study of atmospheric electricity over the ocean, the series extending from the equator southward, did not observe any phenomena which would indicate any unusual ionization of the air by the ultra-violet rays of the sunlight.

Although the spectrum of the sun as shown by the spectrograph does not extend beyond 291 $\mu\mu$, still it may be possible that we receive rays the nature of which we have not yet determined and which, with our present physical technique, we can not determine and which may have an influence in the phenomena of insolation. The discovery of such rays, if they exist, will form an interesting and important chapter in the work on this subject.

[36] *This Journal, Sec. A* (1910), 5, 267.
[37] *Proc. Roy. Soc. London, Ser. A* (1911), 85, 175.

TABLE I.—*Manila. Rate for one hour.*

Month.	Average.	Maximum.	Minimum.	Mean maximum.	Mean minimum.	Average temperature.	Observatory thermometer readings.	Black-bulb readings.	Clear days.
1910.									
May	13.21	17.7	8.21	15.42	6.88	32.09	37.2	46.9	10 out of 27.
June	12.62	17.1	6.08	15.87	10.05	32.85	38.0	47.6	8 out of 25.
July	13.74	17.8	4.61	15.95	10.07	33.37	36.6	47.6	7 out of 24.
August	13.11	17.5	5.11	15.14	10.07	31.27	39.3	49.3	3 out of 25
September	10.94	17.1	1.15	13.51	6.05	30.30	35.2	47.2	4 out of 26.
October	11.78	17.45	1.71	13.89	9.33	30.61	35.6	45.0	4 out of 25.
November	9.97	17.38	1.47	13.78	6.16	28.57	33.8	44.5	1 out of 24.
December	10.03	14.51	1.19	12.44	7.12	29.18	33.1	43.9	3 out of 22.
1911.									
January	12.49	17.04	7.99	14.91	11.20	30.51	35.1	45.3	5 out of 19.
February	11.54	16.32	4.98	14.01	7.97	30.14	34.0	42.0	5 out of 22.
March	13.13	17.35	4.64	15.24	10.45	.....	36.6	46.0	11 out of 25.
April	13.88	17.60	10.49	15.94	12.41	.....	37.4	48.0	6 out of 17.
May	13.27	15.09	10.28	15.48	13.06	.....	39.1	50.3	11 out of 22.
June	14.07	16.38	12.80	15.00	13.42	.....	37.9	49.5	6 out of 17.
July	11.83	14.05	7.11	13.18	10.22	.....	35.7	45.1	0 out of 11.
Average	12.45	16.82	5.52	14.65	9.64	30.869	36.3	46.6	

TABLE II. *Kuala Lumpur. Rate for one hour.*

Month.	Average.	Maximum.	Minimum.	Mean maximum.	Mean minimum.	Clear days.	Temperature.
1911.							
March	15.27	17.3	12.2	16.70	13.56	6 out of 11	31.67
April	15.21	17.5	10.4	16.76	12.79	10 out of 18	32.56
May	15.25	17.5	9.0	16.66	11.25	7 out of 23	32.42
June	15.45	17.3	12.2	16.7	13.97	5 out of 15	32.80
July	14.53	17.3	9.3	15.6	13.09	5 out of 26	32.72
August	15.39	18.1	11.6	16.2	13.62	6 out of 25	32.06
September	15.94	17.5	12.0	16.91	13.99	11 out of 21	31.76
Average	15.29	17.5	11.0	16.52	13.18	.....	32.27

TABLE III.—*Honolulu, Hawaii.*

Month.	Average.	Maximum.	Minimum.	Mean maximum.	Mean minimum.	Average temperature.	Clear days.
1911.							
January	11.78	17.40	3.77	14.67	7.47	21.1	0.
February	13.49	16.71	6.29	15.57	10.72	21.1	7 out of 21.
March	13.82	17.76	3.48	16.26	9.48	21.6	15 out of 25.
April	14.30	17.99	6.46	16.04	10.53	22.8	13 out of 24.
May	13.85	17.04	7.89	15.87	10.62	21.1	9 out of 26.
June	13.64	17.41	6.62	15.46	11.34	21.1	14 out of 25.
July	12.45	16.85	5.53	14.53	9.85	21.1	3 out of 25.
August	14.53	18.51	9.08	16.28	11.92	21.6	10 out of 23.
September	15.30	20.77	6.57	17.07	12.02	21.1	12 out of 20.
October	14.96	18.37	8.24	16.46	12.47	21.1	14 out of 24.
Average	13.81	17.94	6.29	15.82	10.62	21.1	

TABLE IV.—*Tucson, Arizona.*

Date.	Decomposition for one hour.	Temperature.	Remarks.
1910.			
October 24	12.0		
October 25	7.7	32	Thin clouds.
October 26	10.5	23	Overcast, sun 11 to 12.
October 27	13.4	30	Clear, slight cirrus.
October 28	13.2	28	Dust storm.
October 29	12.9	30	Very thin clouds.
October 30	11.1	29	Clear.
Average	11.5	28.7	
Maximum	13.4		
Minimum	7.7		

TABLE V.—*Washington, D. C.*

Months	Average.	Maximum.	Minimum.	Mean maximum.	Mean minimum.	Remarks.	Clear days.
1910.							
June	9.91	12.70	6.54	11.55	7.18		5 out of 8.
July	11.19	15.60	1.70	12.80	7.19		10 out of 14.
August	10.37	15.75	1.85	12.75	6.81		8 out of 10.
September	11.38	19.14	3.83	13.49	8.37		10 out of 18.
October	12.91	16.56	4.37	15.05	10.16		14 out of 16.
November	9.18	15.33	2.66	11.55	6.35	Without temperature coefficient.	4 out of 9.
December	6.61	11.11	3.50	9.85	6.49		8 out of 11.
1911.							
January	9.08	11.50	6.66	10.34	8.07		8 out of 9.
February	12.60	14.24	9.66	13.88	10.68		4 out of 5.
March	11.96	12.63	11.29	12.63	11.29		2 out of 2.
April	13.09	13.48	12.32	13.48	12.32		3 out of 3.
Average	10.58	14.37	5.85	12.48	8.63		
May	7.21	8.21	6.72	7.92	6.27	With our solution.	7 out of 7.

TABLE VI.—*Washington, D. C.*

Months.	Average.	Maximum.	Minimum.	Mean maximum.	Mean minimum.	Remarks.	Clear days.
1910.							
June	9.91	12.70	6.54	11.55	7.16		5 out of 8.
July	11.19	15.60	1.70	12.80	7.19		10 out of 14.
August	10.37	15.75	1.85	12.75	6.81		8 out of 10.
September	11.38	19.14	3.83	13.40	8.37		10 out of 18.
October	12.91	18.66	4.87	15.05	10.16		14 out of 16.
November	11.29	17.33	3.54	14.38	8.82		4 out of 9.
December	11.51	15.85	6.00	14.15	9.27	With temperature coefficient.	6 out of 11.
1911.							
January	12.97	16.42	9.52	14.77	11.53		8 out of 9.
February	15.01	20.34	9.66	20.07	11.64		4 out of 5.
March	11.96	12.63	11.29	12.63	11.29		3 out of 3.
April	13.09	13.48	12.32	13.48	12.82		
Average	11.96	15.99	6.33	14.10	9.60		
May	7.21	8.21	5.72	7.92	6.27	With our solution.	7 out of 7.

TABLE VII.—*Khartoum, Egypt.*

Months.	Average	Maximum	Minimum.	Mean maximum.	Mean minimum.	Maximum temperature	Clear days.
1911.							
September	17.4	19.6	14.8	17.8	16.5	40.2	12 out of 22.
October	17.8	20.8	16.1	18.7	17.3	38.9	22 out of 31.
November	17.4	17.8	16.7	17.7	17.0	35.4	10 out of 13.
Average	17.5	19.4	15.8	18.1	16.9	38.1	

TABLE VIII.—*Baguio. Rate for one hour.*

Months.	Average.	Maximum.	Minimum.	Mean maximum.	Mean minimum.	Observatory thermometer readings.	Black-bulb readings.	Clear days.
1911.								
March	12.5	18.7	6.9	16.2	8.2			5 out of 13.
April	16.1	20.6	8.3	17.9	13.1	27.3	51.2	8 out of 19.
May	16.2	19.4	11.1	17.7	14.0	27.5	51.4	
June	11.9	16.7	7.1					
Average	14.2	18.8	8.3	17.1	11.4			

MUCOCELE AND DIVERTICULUM OF THE VERMIFORM APPENDIX OF INFLAMMATORY ORIGIN.

By B. C. CROWELL.

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While cystic dilatation of the vermiform appendix is in all probability not as rare an occurrence as the literature on the subject would suggest (Kelly, 68 cases), the scant attention accorded it in most text-books on pathology and the paucity of accurate microscopic descriptions which would enable one to form an idea of its pathogenesis amply justify further reports. Feré, in 1887, was the first to apply the term retention cyst, hydrops, or mucocele to that portion of the appendix in which dilatation had occurred, although the condition was first recognized by Virchow, who considered his case as one of colloid degeneration of the appendix.

When the lumen of the appendix becomes occluded at any point, the sequelæ in the distal portion depend upon various factors:

1. If it occur during the course of an acute appendicitis, the remainder of the appendix being also the seat of inflammation, it may result in healing, in perforation, or in gradual general diminution of the lumen of the appendix; that is, the ordinary obliterative appendicitis.

2. If, on the other hand, it occur as the result of a gradually progressing process, a mucocele may result and diverticula may form, the contents being at first mucoid, later clear and watery, and, perhaps, later more viscid and inspissated. For the production of the cystic dilatation, or mucocele, the necessary factors *a priori* are:

(*a*) *Gradual occlusion of one part.*—The cause of this may be a tumor, cicatricial stenosis such as occurs in the healing of ulcers, or angulation produced by peritoneal adhesions, and one case has been reported in which the appendicectomy wound broke down and rapidly healed under antisyphilitic treatment (Lilienthal).

(*b*) *Absence of infection with pyogenic organisms.*—If pyogenic organisms be present at the time of occlusion, or enter

through an incompletely occluded lumen, an empyæma rather than a mucocele may result.

(*c*) *A mucosa able to secrete faster than it can resorb.*—This predicates a functionating mucosa, that is, one in which too extensive pathological changes have not been produced by the causative factor upon which the occlusion depends, and unless secretion proceed more rapidly than resorption, dilatation will not occur. Bierhoff is of the opinion that dilatation is wanting when the mucosa is still able to resorb in the usual way. This latter point has been emphasized by several authors, whereas it would seem that in order to prevent dilatation, resorption through the mucous membrane must proceed more rapidly than under normal conditions, inasmuch as the normal outlet into the cæcum is occluded.

The relative infrequency of mucocele in comparison with other lesions of the appendix is thus seen to be accounted for by the stringency of the requisites for its production or the multiplicity of the factors which must be properly coördinated.

Occlusion under these conditions having been produced, the changes occurring in the distal portion follow in fairly definite order. The accumulation of retained secretion leads to dilatation and degenerative or atrophic changes in the mucosa and submucosa, along with a replacement fibrosis of the tunica muscularis, the fibrosis originating from both the subserous and submucous layers. Some muscular hypertrophy may take place, and if one or more portions of the wall have been unduly weakened by previous disease, for example, ulceration, a diverticulum, or diverticula may result. The changes in the character of the contents depend upon those in the mucosa. A mucoid degeneration of the epithelial elements of the mucosa is a comparatively early feature, leading to over-production of mucus and desquamation of epithelial elements. At this period the contents are thick and mucoid. As the result of the increasing distension, the mucosa undergoes atrophic changes which may involve all or a part of the mucosa according as the previous weakening of the wall has been uniform or otherwise. At the same time the submucosa becomes replaced by fibrous tissue and the contents become clearer and more watery and of the character of a transudate, which may become turbid and more or less viscid from the admixture of cellular elements from the blood and the desquamated epithelium of the mucosa. The pressure may also lead to degeneration of a hyaline or myxomatous character in the fibrous tissue.

The various phases as here depicted form but different stages

in one process, and the result, in whatever stage encountered, has been termed a retention cyst, hydrops, or mucocele. The term retention cyst indicates the origin and is, therefore, correctly applicable to all stages, but it would appear rational to differentiate between a hydrops and a mucocele as being different stages. Where the overproduction of mucus or even definite myxomatous changes predominate, the term mucocele seems more appropriate, while that stage in which the mucosa is destroyed and the cyst filled with a transudate would more appropriately be denominated a hydrops.

It is to be remembered that, according to the position of the occluded portion, the whole or any part of the appendix may be converted into a cyst. Probably the largest described is that of Guttmann, which was 14 centimeters long and 21 centimeters in circumference.

The peculiar character of the contents of a true mucocele along with some unusual features in the epithelial changes have suggested in some cases the possibility of tumor formation. As already mentioned, Virchow considered his case as one of colloid degeneration, and Stengel has reported a case in which he considered the possibility of colloid carcinoma. He also mentions the cases of Rokitansky, Draper, Vimont, and Baillet as possessing histological features suggestive of neoplasms. No case, however, has been recorded, as far as I can ascertain, with indubitable neoplastic features. The extreme mucoid degeneration of the mucosa with desquamation of the epithelium and the myxomatous degeneration of the fibrous tissue form a picture readily mistaken for a tumor, without the actual presence of tumor formation. I believe it to be a retrogressive rather than a progressive metamorphosis.

Congénital diverticula of the appendix have been described, especially by Hedinger, but the chance of as rare a lesion as a mucocele occurring in an appendix with a congenital diverticulum seems remote; on the other hand, in a mucocele all the factors necessary for the formation of acquired diverticula may be at hand.

The case here to be reported (number 1408) was discovered in the necropsy of a male Filipino, 40 years old, who had died of pulmonary tuberculosis, and in whom there was no evidence of abdominal tuberculosis.

The vermiform appendix was 4.5 centimeters long, occupied its normal position, and there were no surrounding adhesions. At its origin from the cæcum on the side opposite the mesentery

was a globular swelling 2 centimeters in diameter and raised 1.5 centimeters above the surface of the appendix. This swelling was covered with peritoneum continuous with that clothing the appendix, its surface was perfectly smooth, and the mass was slightly fluctuating. The tip of the appendix seemed to have a somewhat thinner wall than the remainder of the organ, and the intermediate portion was thicker than normal. These features are illustrated in Plate I, fig. 1, a photograph taken after opening the appendix and removal of some of the contents, thus accounting for the shrunken appearance of the cyst. The appendix, cyst, and adjacent portion of the cæcum were preserved *in toto*. On opening the appendix longitudinally (after fixation), the picture as seen in Plate I, fig. 2, was disclosed. The hypertrophic base of the appendix projected a few millimeters into the lumen of the cæcum much as the cervix uteri projects into the vagina; its orifice was practically occluded, allowing the passage of only a very fine strand of catgut. The proximal and distal thirds of the appendix were dilated, while the middle third was almost obliterated by the approximation of its walls. External to the proximal half of the appendix was the cyst, the inner wall of which was formed by the muscular tunic and the outer wall by a thin membrane covered by peritoneum. No direct communication of this cyst with the lumen was discovered on gross examination. The cyst and the proximal and distal thirds were distended by a thick, pearly-white, translucent mucoid material of about the consistence of gelatine, showing flecks of a whiter substance embedded in it.

Description of the microscopic details must include a study of practically the entire appendix, and for the purpose of clear orientation the different localities will be studied separately.

1. The proximal portion of the appendix. This shows a marked inflammatory hypertrophy which has led to the projection of the appendix into the cæcum, and the practical obliteration of the lumen of this part of the appendix. The inflammatory condition is manifested by fibrosis with round-celled infiltration and a muscular atrophy, along with a marked mucoid degeneration of the mucosa. The glandular tubules of the mucosa are separated by masses of round cells and are probably diminished in number, whereas the individual epithelial cells of the glands are almost entirely transformed into large goblet cells with basal nuclei surmounted by large cup-shaped cavities just emptied of their mucus contents. This condition exists for about 4 millimeters within the appendix.

2. Just distal to this there is a considerable dilatation of the lumen for a distance of from 7 to 8 millimeters. This area shows some thickening of the wall of the appendix on the mesenteric side, the thickening being accounted for chiefly by an increase of the fibrous elements of the submucosa along with some muscular hypertrophy and slight increase of the subperitoneal fibrous tissue. The mucosa here has largely disappeared, and in places the fibrous submucosa shows a myxomatous degeneration.

On the side opposite the mesentery, however, the wall is much thinner, and it is from this portion that the cyst has arisen. From within outward, the mucosa is largely destroyed, the submucosa fibrous, the muscularis is much atrophied, and one portion of the subperitoneal fibrous tissue is slightly thickened. At one point the mucosa, submucosa, muscularis, and a portion of the subperitoneal fibrous tissue have undergone a solution of continuity, and the cyst has formed as a hernial protrusion of the peritoneum, lined internally by a thin part of the outer layer of subperitoneal fibrous tissue. Direct connection exists between the cyst and the lumen of the appendix at this point, which is 15 millimeters from the base of the appendix. The fibrous tissue lining this cyst has undergone extensive myxomatous degeneration.

3. Distal to this, the lumen of the appendix is reduced to a minimum, the reduction being caused by a much thickened and fibrous submucosa and muscularis encroaching upon the lumen. Here the mucosa is represented by only an occasional remnant of a glandular tubule.

4. Beyond this the distal third of the appendix is dilated and its walls are thin. Here remnants of mucosa remain in a state of mucoid degeneration with abundant round-celled infiltration, the lymphoid elements of the submucosa are replaced by fibrous tissue, the muscularis is atrophied, and the subperitoneal fibrous tissue is somewhat thickened. The extreme apex is formed by a thin wall of fibrous tissue containing only a few muscular bundles.

The contents of the cyst and appendix are of a myxomatous character, the white flakes mentioned corresponding to masses of exfoliated epithelium and cells which have exuded from the vessels. These masses do not take the form of definite glandular structures, but in places do suggest that they are portions of the mucosa which have become exfoliated. They are prob-

ably of the same nature as those described in Stengel's case and do not, to my mind, furnish evidence of tumor formation. The number of leucocytes associated with the exfoliated epithelium is remarkable. In no place in the appendix is there anything suggesting invasion of the muscularis by epithelial elements. In the appendix where the mucosa is lacking, the lining fibrous tissue has undergone a myxomatous transformation, being represented by but few cells and abundant transparent, homogeneous, intercellular substance. In the wall adjacent to the point of formation of the cyst there is evidence of an old hæmorrhage.

This case exemplifies one mode of formation of both mucoceles and diverticula. The preexisting inflammation, upon which the occlusion of the orifice depended, was also responsible in all probability for some of the destruction of the mucosa as well as for the almost complete destruction of the wall at one point which made possible the formation of the cyst. The degenerative and atrophic changes were natural sequelæ. Had the constriction in the middle third been more nearly impermeable, then a second cyst would have developed involving the distal third. Had the condition persisted for a greater length of time, the character of the contents would have been changed, the wall would have become thin and transparent, and the cyst much larger.

Plate 2 is a semidiagrammatic drawing of a section stained by Van Gieson's method, designed to show the method of formation of the diverticulum as well as the mucocele.

SUMMARY.

1. A mucocele of the appendix is a retention cyst, the requisites for the production of which are:
   a. Gradual occlusion of the lumen.
   b. Absence of infection with pyogenic organisms.
   c. A mucosa able to secrete faster than it can resorb.
2. The infrequency of the association of these factors accounts for the rarity of mucoceles.
3. They are of inflammatory origin, no case indubitably of intrinsic tumor formation having been recorded.
4. The terms hydrops and mucocele are used interchangeably, but it would seem preferable to apply the terms to different stages of the same process.
5. Diverticula of the appendix not infrequently occur in the formation of mucoceles.

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For a more complete bibliography, the reader is referred to the articles of Kelly and Stengel.

ILLUSTRATIONS.

PLATE I.

FIG. 1. Appendix and diverticulum after incision and partial removal of contents. Portion of cæcum also shown. (Photograph by Cortes.)

2. Opened appendix and diverticulum. (Photograph by Cortes.)

PLATE II.

Semidiagrammatic drawing of a section of appendix stained by Van Gieson's method.

1. Oblique section of practically occluded lumen at origin.
2. Wall of cæcum.
3. Peritoneum.
4. Diverticulum.
5. Subserous fibrous tissue.
6. Submucous fibrous tissue.
7. Muscularis.
8. Remnants of mucous membrane.
9. Point immediately adjacent to opening of diverticulum.

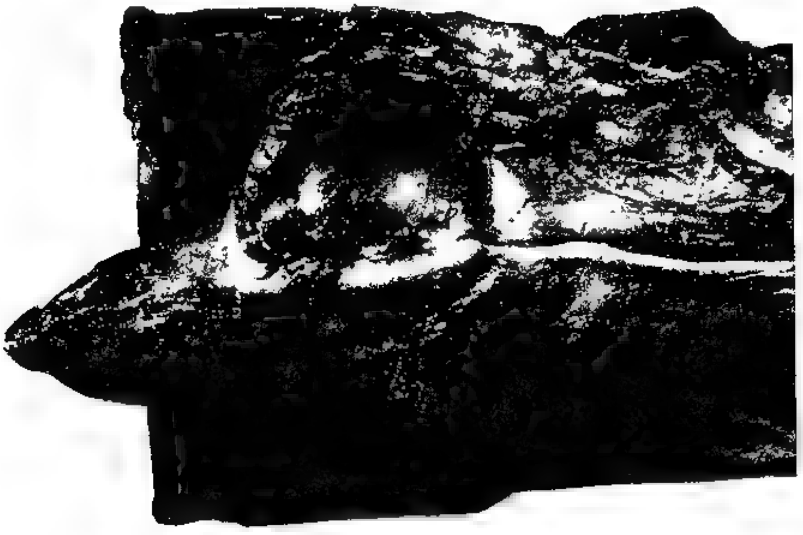
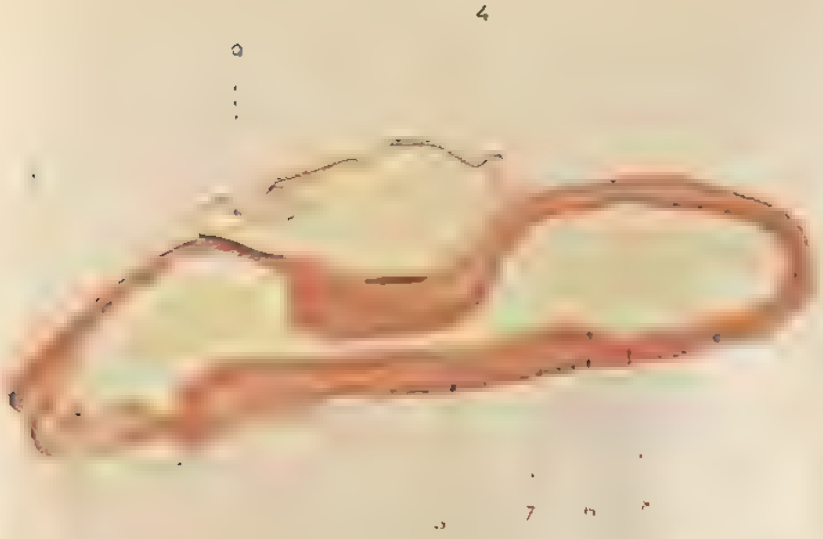


Fig. 1. Appendix and diverticulum.

Fig. 2. Opened appendix and diverticulum.

PLATE I.



SECTION OF APPEND X STAINED BY VAN GIESON'S METHOD

PLATE II

A THIRD CONTRIBUTION TO THE ETIOLOGY OF BERIBERI.[1]

By

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In previous communications (1) (2) Chamberlain and Vedder showed that the substance in rice polishings which prevents polyneuritis gallinarum was present in an extract of the polishings having the composition shown in Table I.

TABLE I.—*Analysis of a neuritis-preventing extract of rice polishings.*

Constituents.	Per cent.
Total solids	1.34
Ash	0.03
Phosphorus pentoxide	0.00165
Nitrogen	0.0406
Sucrose	0.88

It was further proved that the neuritis-preventing principle was soluble in cold water, cold 95 per cent alcohol, was dialyzable, and was adsorbed by bone black or animal charcoal. By experiments on fowls the phosphorus, the inorganic salts, and the sucrose of this extract were excluded from further consideration as regards the prevention of neuritis.

We had hoped that the problem could next be attacked by direct chemical analysis, but a preliminary examination showed that the extract was of so complex a nature as to render a direct search for the neuritis-preventing substance impracticable. Therefore a further study of the nitrogenous constituents of this extract was begun.

For this purpose a fresh supply of extract of rice polishings

[1] Published with permission of the Chief Surgeon, Philippines Division.

[2] W. P. Chamberlain, major, Medical Corps, United States Army, and E. B. Vedder, captain, Medical Corps, United States Army, members of the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands. R. R. Williams, chemist in the Bureau of Science, Manila, P. I. The chemical work relating to amido-bodies was performed by Mr. Williams. This paper was submitted for publication in September, 1911.

was prepared according to the method previously described (1), except that the polishings were extracted by three successive macerations with fresh 95 per cent alcohol and that the residue obtained by this extraction of 10 kilograms of polishings was redissolved in 1 liter of distilled water instead of the usual 10 liters. These two changes of method were made in order to obtain a more concentrated extract which would be better suited for chemical analysis. The extract used in the following experiments, therefore, is several times stronger than that previously used. The results of its analysis are shown in Table II.

TABLE II.—*Analysis of a neuritis-preventing extract of rice polishings, made more concentrated than that shown in Table I.*

Constituents.	Per cent
Specific gravity	1.0437
Total solids	12.517
Sucrose [a]	6.33
Reducing sugars	0.52
Total nitrogen	0.161
Amido-nitrogen	0.156
Phosphorus pentoxide	0.006
Ash	0.27
Undetermined	5.23

[a] The determination of sucrose was not accurate because of the presence of other optically active substances.

Nonproteid nitrogenous substances are commonly found in seeds and in other parts of plants. Thus, in *Lupinus luteus*, Schulze (4) found asparagin, phenyl-alanin, amido-valeric acid, arginin, cholin, and xanthin like substances; in *Cucurbita pepo*, glutamin, asparagin, leucin, tyrosin, arginin, cholin, vernin, and xanthin-like substances; in *Vicia sativa*, asparagin, phenyl-alanin, leucin, amido-valeric acid, guanidin, cholin, and betain. Of these, asparagin, glutamin, and arginin occur most widely and plentifully.

The amount of amido-nitrogen present in rice, rice polishings, beans, and *mongos* was found to bear a very close relation to the amount of phosphorus pentoxide present in those substances, as may be seen from Table III.

TABLE III.—*Relationship between percentage of nitrogen and amido-nitrogen in rice and beans.*

Article.	Total nitrogen.	Amido-nitrogen
	Per cent.	*Per cent.*
Highly milled rice, No. 1	1.48	0.03
Highly milled rice, No. 2	1.22	0.00
Rice polishings, No. 1	5.40	0.96
Rice polishings, No. 2	5.10	0.504
Navy beans	4.13	1.23
Mongos	3.71	0.616

The amido-nitrogen is low in the highly milled rices which are always low in phosphorus pentoxide; and high in rice polishings, beans, and *mongos* which contain a considerable proportion of phosphorus pentoxide. This is an interesting parallel to the work of Parrozzani(3) who showed that the amido-nitrogen and total nonproteid nitrogen in many grains commonly used as food are both proportional to the content of organic phosphorus. The fact is also interesting in the consideration of a suitable index of the beriberi-preventing character of certain rices. The amount of phosphorus pentoxide has hitherto been used as such an indicator with general satisfaction, but we have recently examined samples of undermilled rices which retained almost the entire pericarp and which undoubtedly will prevent beriberi, and yet these rices on analysis were shown to contain a percentage of phosphorus pentoxide as low or lower than that in some specimens of highly polished rice which have been analyzed by the same chemists. There can be no doubt that the character of the soil may effect very materially the phosphorus content of rices as it does in the case of other grains. Chamberlain, Bloombergh, and Kilbourne(5) have shown that the percentage of potassium present in a rice is satisfactory as an indicator, and it now appears that the amount of amido-nitrogen may also be equally reliable.

These observations pointed to the possibility that some nonproteid nitrogenous substance, like arginin or asparagin, might be the neuritis-preventing substance which is present in rice polishings. As is well known, Takaki considered the disappearance of beriberi from the Japanese navy to be due to the increase in nitrogenous constituents in the ration. Although this theory of nitrogen starvation has long been discredited, the possibility that there might be a deficiency in a particular nitrogenous compound, such as amido-nitrogen, has not to our knowledge been considered. This possibility was further emphasized by a study of two dietaries used in Bilibid prison in Manila(6). The first ration (Table IV) was in use from about December 1, 1901, until October, 1902, during which time there were 5,448 cases of beriberi with 229 deaths. Beriberi completely disappeared after the institution of the second ration (Table V). The two rations are given in full, together with their proximate principles, and it will be seen that while there was no appreciable change in the amount of albuminates in the two dietaries, the amount of amido-nitrogen, calculated as asparagin, was only 5.84 in the first ration and was increased

to 12.26 in the second. Aside from the increase in amido-nitrogen there was no marked change perceptible except that the amount of carbohydrate was greatly reduced in the second ration.

TABLE IV.—*Ration for convicts at Bilibid from December 1, 1901, to October, 1902; 5,448 cases of beriberi occurred in this period.*

Ration.		Proximate principles.[a]					
Components.	Amounts.	Non-proteid nitrogen.	Albumin-ates.	Proteid nitrogen	Fats.	Starches.	Salts.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Sugar	28.35					27.35	0.14
Bread	151.20	0.38	12.09	1.55	2.26	74.39	1.96
Rice	453.60	0.09	35.43	5.58	3.12	377.40	2.25
Beef	226.80	0.27	48.52	7.49	11.77		3.62
Potatoes	85.05	0.34	1.13	0.18	0.09	11.90	0.50
Onions	28.35	0.28		0.09			
Pepper	0.50						
Vinegar	10.00						
Salt	18.00						18.00
Ginger root	28.35						
Total		1.36	97.17	14.89	17.24	491.04	26.48
Total vegetable amids, calculated as asparagin, 5.84 grams							

[a] The proximate principles as given in the tables are taken from the tables in the article by Fales(6), except the proteid and nonproteid nitrogen, these having been estimated by Mr. Williams.

TABLE V.—*Ration for convicts at Bilibid introduced in October, 1902. Beriberi disappeared after this change.*

Ration		Proximate principles					
Components.	Amounts.	Non-proteid nitrogen.	Albumin-ates.	Proteid nitrogen.	Fats.	Starches.	Salts.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Sugar	28.35					27.35	0.14
Bread	302.40	0.76	24.18	3.11	4.53	148.78	3.93
Rice	255.15	0.05	17.71	2.78	1.56	188.70	1.13
Beef	226.80	0.27	48.52	7.49	11.77		3.62
Dried fish	56.70	0.15	7.08	0.97	0.85		0.28
Potatoes	119.07	0.48	2.38	0.37	0.14	25.00	1.19
Onions	102.06	1.00	1.84	0.29	0.50	5.90	0.70
Pepper	0.50						
Vinegar	10.00						
Salt	18.00						18.00
Ginger root	28.35						
Total		2.71	101.71	15.01	19.35	395.73	29.99
Total vegetable amids, calculated as asparagin, 12.26 grams.							

The extract of rice polishings considered in Table II was, therefore, submitted to chemical treatment in order to divide the amid substances into classes and to enable us to test each class by means of experiments on fowls. The following is the process which was employed.

In order to remove noncoagulable proteids and other colloidal substances, the extract obtained from 3 kilograms of rice polishings was treated with an excess of basic lead acetate, the precipitate was removed by filtration, washed with water, and redissolved in 40 cubic centimeters of 10 per cent sodium hydroxide solution. The lead in this solution was precipitated with sulphuretted hydrogen and filtered. The filtrate was rendered slightly acid with hydrochloric acid (whereupon the substances originally precipitated by the basic lead acetate were once more thrown down), was allowed to stand over night in order that the excess of sulphuretted hydrogen might be dissipated, and the following day was rendered slightly alkaline for the purpose of again dissolving the precipitate. The solution was then diluted to 3 liters so that each cubic centimeter of solution represented the substances precipitated by basic lead acetate from the extract of 1 gram of polishings. Ten cubic centimeters of this solution were given to fowls daily, and it was denominated Extract Number 24.

The filtrate remaining after the lead acetate precipitate had been removed was treated with a slight excess of sulphuric acid to remove the lead. The lead sulphate thus formed was filtered. A saturated solution of phospho-tungstic acid was added to the filtrate until precipitation was complete. (About 10 grams of phospho-tungstic acid were required.) The rather bulky, orange-yellow precipitate was removed and mixed with freshly slaked lime and barium hydroxide. The lime unites with the phospho tungstic acid leaving in solution free bases of the histon group, including arginin. The excess of lime and baryta was removed by precipitation with carbon dioxide and the solution was neutralized with hydrochloric acid, a few drops of sulphuric acid being added to precipitate any barium salt remaining. The filtrate was diluted to 3 liters. The solution thus prepared represented the free bases of the histon group, chiefly arginin and histidin, extracted from the rice polishings, each cubic centimeter being equivalent to 1 gram of polishings. Ten cubic centimeters were given daily to fowls, and it was denominated Extract Number 25.

The filtrate remaining after the separation of the phospho-tungstic acid precipitate contained amino-acids and other substances. In order to remove the excess of phospho-tungstic acid, barium hydroxide was added to slight excess and barium phospho-tungstate filtered off. Sulphuric acid was added until the solution was slightly acid and the barium sulphate removed. The filtrate, containing amino-acids and amino-acid amids, was likewise diluted to 3 liters and fed to fowls as Extract Number 26.

Experiment 10.[3]—Four fowls were fed on polished rice plus a daily dose of 10 cubic centimeters of Extract Number 24,

[3] Experiments 1 to 9, inclusive, are recorded in the two previous articles referred to under references (1) and (2).

containing the precipitate obtained by basic lead acetate and dissolved after removing the lead with hydrogen sulphide.

One fowl developed neuritis in twenty-five days, 1 in twenty-eight days and 1 in twenty-nine days, after which the experiment was discontinued.

Experiment 11.—Four fowls were fed on polished rice plus a daily dose of 10 cubic centimeters of Extract Number 25, containing the histon bases.

One fowl developed neuritis in twenty days and 1 in twenty-eight days, after which the experiment was discontinued.

Experiment 12.—Four fowls were fed on polished rice plus a daily dose of 10 cubic centimeters of Extract Number 26, containing amino-acids.

Two fowls developed neuritis in twenty-six days and 1 in thirty-one days, after which the experiment was discontinued.

In order to check these experiments we also fed fowls with pure chemical substances of the amino-acid group and with amids. Glycocoll, asparagin, and succinamid were chosen as representing the general classes of amino-acid and amid substances likely to be present in the extract of rice polishings. Thus glycocoll or amino-acetic acid contains an amino radical attached to a carbon atom. Asparagin or amino-succinamic acid contains 1 amino and 1 amid group (NH_2), while succinamid contains 2 amido groups.

Experiment 13.—Four fowls were fed on polished rice plus a daily dose of 10 cubic centimeters of a 0.5 per cent suspension of glycocoll in water.

One fowl developed neuritis in thirty days and 1 in thirty-five days, after which the experiment was discontinued.

Experiment 14.—Four fowls were fed on polished rice plus a daily dose of 10 cubic centimeters of a 0.5 per cent solution of asparagin in water.

One fowl developed neuritis in thirty-two days and 1 in thirty-three days, after which the experiment was discontinued.

Experiment 15.—Four fowls were fed on polished rice plus a daily dose of 10 cubic centimeters of a 0.5 per cent solution of succinamid in water.

One fowl developed neuritis in twenty-nine days and 1 in thirty-one days, after which the experiment was discontinued.

The doses of these substances used in the above experiments were selected because it was certain from the chemical analysis that the amount of rice polishing which is known to be sufficient

to protect a fowl from polyneuritis could not contain more than these quantities of amido substances.

It will be seen that all of the experiments were negative, and showed that none of these substances possessed any protective power. Although these experiments do not exclude all such substances which are present in the extract of rice polishings, it seems improbable that the remaining ones can be of importance.

An objection may be raised to part of these experiments because of the fact that the extract was treated with alkaline reagents. Fraser and Stanton(7) have shown that treatment with sodium hydrate destroys the neuritis-preventing substance. We have repeated this experiment and have found that treatment of the extract with sodium hydrate does remove its protective action. However, it should be remembered that the chemical procedure which we followed, as described above, is the recognized method for the isolation of the histon bases; therefore, whatever effect the alkaline reagents may have had on the neuritis-preventing substance, they could not have affected such substances as arginin or histidin. That the neuritis-preventing substance may be destroyed by such treatment is simply another argument against the possibility that any protective action is possessed by the compounds in question.

Since the extract used in the above experiments was a different lot from that employed in the work previously reported by us(1) (2), a control experiment was performed in order to be certain that the extract would prevent neuritis before it was put through the chemical processes already described. For this purpose the concentrated extract was diluted in such proportion that 1 cubic centimeter represented the substances dissolved from 1 gram of rice polishings. Four fowls were then fed on polished rice with a daily dose of 10 cubic centimeters of this diluted extract. These fowls all remained well for fifty days, when they were released. This definitely shows that the neuritis-preventing substance was present in the extract previous to its chemical treatment.

We now considered that it might be of interest to determine exactly what dose of this extract was required in order to prevent neuritis and for this purpose performed Experiment 16.

Experiment 16.—Four fowls were fed on polished rice plus a daily dose of 5 cubic centimeters of the extract of rice polishings. These 4 fowls also remained well for fifty days when they were released. Four fowls were then fed on polished rice plus a daily dose of 2.5 cubic centimeters of the extract of rice

polishings. Two fowls developed neuritis on the fortieth day and 1 developed neuritis on the forty-first day. The fourth fowl remained well for fifty days when he was released.

It is, therefore, obvious that 2.5 cubic centimeters of our extract, equivalent to 2.5 grams of the rice polishings, is insufficient to protect the majority of fowls as long as fifty days, although delaying the onset of symptoms of neuritis for about ten days, but that 5 cubic centimeters is sufficient to afford complete protection for at least fifty days. The fact that a definite dose of this extract is necessary to protect fowls, while a smaller dose delays the onset of the disease, is an additional argument in favor of the assumption that polyneuritis gallinarum is a disease due to the absence of some essential nutritive principle. This work with reduced doses of extract agrees quantitatively with the results obtained by Fraser and Stanton, who found that 5 grams of rice polishings were sufficient to prevent neuritis when fowls were subsisting on polished rice.

We have long been convinced that polyneuritis gallinarum is due to a nutritive deficiency, but apparently some others are not, since Kohlbrugge has recently published an article maintaining that this disease is caused by the acid produced during the fermentation of rice which results from the action of a number of acid-producing bacteria contained in the rice itself. There are many possible criticisms of Kohlbrugge's work, but we will limit ourselves to the following:

Kohlbrugge states that he has produced beriberi in fowls in four or five days by feeding polished rice mixed with agar cultures of the acid-producing bacteria isolated from rice. However, he did not demonstrate the existence of nerve degenerations, and gave few details of his experiment. From what we know of beriberi and polyneuritis gallinarum it seems to us impossible that he could produce this disease in four or five days, and it appears that his fowls must have died of toxæmia or infection, and not of beriberi. He further states that the disease is prevented by large amounts of acid in the food, maintaining that excessive acidity inhibits the growth of the bacteria. Thus rice polishings, beans, etc., are preventive because they contain a great amount of acid. This is certainly incorrect, because we have prevented neuritis in fowls by means of an extract of rice polishings that had been *neutralized* with sodium carbonate. It may be possible that some particular acid, or its salt, is the neuritis-preventing substance, but we have tried phosphoric acid, sulphuric acid, and lime juice with negative results. Since

these three acids failed to prevent neuritis and since a neutral extract added to polished rice did prevent the disease, it is quite obvious that acidity *per se* can be of no importance.

The following points are of interest with reference to Kohlbrugge's view that a culture of his rice bacillus, obtained from fermented rice, was capable of producing neuritis of fowls in four or five days.

Shiga(9), in endeavoring to test the validity of the intoxication theory, performed the following experiment: He fermented an undermilled rice in the incubator for about a week and fed fowls on this fermented material, but they remained healthy for two hundred days, when the experiment was discontinued. We have repeated this experiment with the same result. We have, also, fed fowls on a highly milled rice, fermented in a similar way, and have found that these fowls do not develop neuritis any sooner than fowls fed on the same rice when perfectly fresh and dry. These facts seem to us absolutely to disprove Kohlbrugge's theory.

On comparing the two rations used in Bilibid prison (Tables IV and V), it will be seen that in the latter dietary (which led to the disappearance of beriberi) one of the most obvious changes was the very great increase in the amount of onions prescribed. Onions are very rich in amido-nitrogen compounds, and the oil of onions is composed almost entirely of allyl sulphide $(C_3H_5)_2S$. In order to determine whether the increased consumption of onions could have been responsible for the disappearance of beriberi, Experiment 17 was performed. Two kilograms of onions were ground up finely in a meat cutter, mixed with 2 liters of water, and allowed to macerate for twenty-four hours in the ice-box. The mixture was then filtered. The clear filtrate contained the greater bulk of the onions, very little solid matter being left on the filter.

Experiment 17.—Four fowls were fed on polished rice plus a daily dose of 10 cubic centimeters of extract of onions.

One fowl developed neuritis in twenty-one days, 1 in twenty-two days and 1 in forty-three days, after which the experiment was discontinued.

Since the neuritis preventing substance is soluble in water, it should have been contained in this extract if it were originally present in the onions. As the experiment was negative, we are forced to conclude that onions will not prevent polyneuritis gallinarum, and therefore are not likely to prevent beriberi.

The only other differences that are apparent between the second and first rations at Bilibid are (*a*) an increase in potatoes from 85.05 grams to 119.07; (*b*) a decrease in rice from 458.60 grams to 255.15 grams; and (*c*) an increase of bread from 151.20 to 302.40 grams. It seems improbable that the small increase in potatoes could have been of importance. However, the amount of rice consumed was decreased about one-half, and the amount of bread consumed was doubled, and this simple change was, in our opinion, the one that put an end to the very serious epidemic of beriberi.

A number of instances where similar slight changes have resulted in the prevention of beriberi are on record in the literature of the subject, among which we may mention the following:

Beriberi was very prevalent among the Japanese soldiers at Port Arthur during the Russo-Japanese war. They received a daily ration of 30 ounces of rice and 5 ounces of meat. The Japanese sailors on the other hand, serving on shore and living under similar circumstances except as to their diet, did not have beriberi. They received a daily ration of 20 ounces of rice, 10 ounces barley, and 1 pound of meat. In this case, therefore, beriberi was prevented by the reduction in the amount of rice consumed, the addition of barley, and an increase in the meat component. By the simple change involved in the reduction of the amount of rice consumed, and the addition of a legumen to the diet, beriberi was reduced in the Philippine Scouts (native) from an average of 600 cases a year to *nil*. The instances where beriberi has been prevented by the substitution of undermilled (or cured) rice for highly milled (or polished) rice are too well known and numerous to mention. Although the discovery of the actual neuritis-preventing substance is of the greatest scientific interest and importance, we consider, in view of the facts already known and referred to above, that the hygienic problem of the prevention of beriberi is already solved.

In the further chemical analysis of the extract of rice polishings, a substance thought to be cholin was isolated. This also seemed worth testing, since cholin is an important constituent of nerve tissue, combined with phosphorus and fatty acids in the form of lecithin. Moreover, cholin fulfils the chemical and physical requirements which we have experimentally determined that the neuritis-preventing substance possesses. On account of the difficulty in obtaining pure cholin we used lecithin made from eggs (Merck's preparation).

Experiment 18.—Four fowls were fed on polished rice plus a daily dose of 0.3 gram lecithin.

One fowl developed neuritis in eighteen days and 1 in twenty-four days, after which the experiment was considered negative and discontinued. Since in chemical character lecithin is a salt containing cholin as its base, we conclude that this experiment

excludes cholin and lecithin from further consideration as the neuritis-preventing substance of rice polishings.

While our observations had led us to believe that the neuritis-preventing principle in rice polishings was insoluble in ether, there was no conclusive evidence with regard to this point. To settle the matter, polishings were extracted with ether as follows:

Two kilograms of polishings were mixed with 6 liters of ether and, after macerating for twenty-four hours, the ether extract was filtered off. The extraction was repeated with fresh ether for a second twenty-four hours, the filtrates combined, and the ether evaporated by the current from an electric fan. Three hundred cubic centimeters of residue, consisting chiefly of fat, were obtained from 2 kilograms of polishings.

Experiment 19. —Four fowls were fed on polished rice plus a daily addition of 2 cubic centimeters of the ethereal extract of rice polishings. One fowl developed neuritis in twenty-one days, 1 in twenty-nine days, and 1 in thirty days. From this experiment we concluded that the neuritis-preventing principle is insoluble in ether.

We have never seen œdema in any of the fowls that have developed polyneuritis, although this symptom has been noted by several other observers, including Shiga(9). The influence of a salt-free diet in reducing the œdema of nephritis has been much emphasized of late. Since the fowls fed on highly milled rice receive practically no salt, it seemed possible that the addition of a considerable quantity of sodium chloride to the food might produce this symptom. In order to test this hypothesis Experiment 20 was performed.

Experiment 20.—Four fowls were fed on polished rice, and in addition were given a daily dose of 1 gram of sodium chloride diluted in 10 cubic centimeters of distilled water. This would be equivalent to a dose of 50 grams daily for a man. The 4 fowls developed neuritis on the twenty-third, twenty-sixth, twenty-seventh, and thirty-third days, respectively, but at no time did they show any signs of œdema. Therefore, we concluded that the presence or absence of sodium chloride is of no importance with regard to the development of œdema in polyneuritis gallinarum and probably in beriberi.

In a previous paper(2) we reported an experiment demonstrating that the neuritis-preventing principle was adsorbed when the extract of rice polishings was passed through an animal charcoal filter, and that a portion of the neuritis-preventing element was recovered when the bone black from the filter was washed with distilled water. Unfortunately, the charcoal used

in the above experiment was not chemically pure. Consequently we were unable to obtain any information as to the chemical nature of the substances removed from the extract of rice polishings by this method.

Experiment 21.—Therefore, a second experiment was performed in the following manner: The extract obtained from 10 kilograms of polishings was dialyzed, according to the method described in our previous paper, and the diffusate was then filtered through animal charcoal (Kahlbaum I). The filtrate was clear and colorless. This filtrate was then administered to 4 fowls that were being fed on polished rice. Two of these fowls developed neuritis on the twenty-fifth day, 1 on the twenty-eighth, and 1 on the thirty-fifth day. This showed conclusively that the neuritis-preventing substance remained behind in the charcoal, thus confirming the results obtained in our previous experiment.

The charcoal was then transferred from the filter to a flask and was mixed with 500 cubic centimeters of ether. The mixture was allowed to stand in the ice box for two days, being repeatedly shaken during this time. The ether was then filtered off and evaporated, and the ethereal extract so obtained was redissolved in water. Four fowls were fed on highly milled rice and given in addition a daily dose of this extract. One fowl developed neuritis on the twentieth day, 1 on the twenty-third day, 1 on the thirty-eighth day, and 1 on the forty-second day. Therefore, it was apparent that the neuritis-preventing principle was not removed from the charcoal by the ether.

The charcoal was then treated in a similar manner with 1 liter of absolute alcohol. The alcoholic extract obtained was of a yellowish tinge and distinctly acid to litmus. The alcohol was evaporated and the residue redissolved in water as in the case of the ethereal extract. Four fowls were fed on highly milled rice plus a daily dose of this extract removed from the charcoal by alcohol. One fowl developed neuritis on the twenty-third day, 1 on the thirty-second day, 1 on the thirty-seventh, and 1 fowl remained well for sixty-one days. As a result of this experiment it is clear that the neuritis-preventing substance was not removed from the charcoal by absolute alcohol.

The charcoal was then washed with 8 liters of distilled water, shaking the charcoal up with successive portions of water and permitting the extraction to last for ten days. The water recovered from the charcoal was also acid to litmus. Four fowls were fed on highly milled rice plus a daily addition of this

watery extract from the charcoal. One fowl developed neuritis in twenty-three days, 1 in twenty-six days, 1 in thirty-six days, and 1 remained well at the end of forty-eight days. Therefore, it appeared that the neuritis-preventing principle was not removed, by extraction with water, from the pure charcoal used in this experiment.

The charcoal was next suspended in 500 cubic centimeters of distilled water, and 2 cubic centimeters of this mixture fed daily to 4 fowls subsisting on higly milled rice. Since the charcoal rapidly sinks to the bottom, the flask was shaken just before giving the mixture. The water was used simply as a vehicle for the administration of the charcoal. These 4 fowls all remained well at the end of sixty days. Therefore, we concluded that the neuritis-preventing principle was still retained by the charcoal in spite of the repeated extractions to which it had been subjected. This experiment differed from the one reported previously in that water completely failed to remove the neuritis-preventing principle, while in the former experiment (Number 6) this principle seemed to be in part removed, and the watery extract conferred partial protection. We attribute this difference in results only to the fact that in the first instance we used an impure charcoal, while the second time we used a pure product which apparently possessed a greater adsorptive power for the unknown neuritis-preventing substance.

Experiment 21 fully confirms our former observation that the neuritis-preventing principle in rice polishings is adsorbed by animal charcoal and this knowledge perhaps will be the basis for a method of isolating the substance in comparative purity, provided a means can be discovered for extracting it from the charcoal.

CONCLUSIONS.

1. These experiments all substantiate the theory that polyneuritis gallinarum and beriberi are caused by the deficiency of some as yet unknown substance in the food. We have shown previously that this substance is not phosphorus.

2. Kohlbrugge's theory that beriberi is caused by an acid intoxication, which is due to the fermentation of rice by various saprophytic bacteria contained in the kernel, must be regarded as untenable.

3. To the list of substances which we have shown in previous papers to be of no importance in preventing neuritis of fowls there may now be added the following: Nitrogenous compounds

such as arginin, histidin, asparagin, and various amino-acids; lipoids of the lecithin group and cholin; extract of onions.

4. The neuritis-preventing principle is insoluble in ether.

5. The neuritis-preventing principle is adsorbed by animal charcoal and the filtrate through the charcoal will not prevent neuritis. After adsorption the active principle can not be removed from the charcoal by maceration with water, absolute alcohol, or ether.

6. The administration of large quantities of sodium chloride failed to produce œdema in fowls suffering from polyneuritis.

7. Five cubic centimeters of our extract (equivalent to 5 grams of rice polishings) is sufficient to protect fowls subsisting upon polished rice. Two and one-half cubic centimeters (equivalent to 2.5 grams of polishings) is insufficient to confer complete protection against polyneuritis.

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THE SCHIZOGONY OF TRYPANOSOMA EVANSI IN THE SPLEEN OF THE VERTEBRATE HOST.

By ERNEST LINWOOD WALKER.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

There have been in recent years observations by several investigators that indicate a more complicated developmental cycle of trypanosomes in the vertebrate host than has hitherto been suspected.

Salvin-Moore and Breinl, in 1907, described the development of round bodies from the trypanosomes in the lungs of rats infected with *Trypanosoma gambiense*. These bodies are formed at or near the maximum of the multiplication of the trypanosomes in the circulating blood. In their development, there is, according to these authors, first an interaction between the blepharoplast ("extra-nuclear centrosome") and the nucleus of the trypanosome in the form of a stainable band that grows out from the blepharoplast and becomes connected with the nucleus. This band later becomes broken up and disappears, but it is believed that during the process a part of the blepharoplast becomes united with the nucleus. The protoplasm of the trypanosome then becomes separated from the nucleus which lies in a vaculoid space. This vesicle, containing the nucleus and surrounded by a thin layer of protoplasm, becomes detached from the body of the trypanosome which disintegrates. These bodies are stored chiefly in the spleen and bone marrow, and are considered by Salvin-Moore and Breinl as a resistant stage of the trypanosome which persists when the parasites disappear from the peripheral blood and which give rise by development to the new generation of motile trypanosomes that reappear in blood after the lapse of a variable period of time. For this reason these bodies are designated by these authors as "latent bodies."

Chagas, in 1909, published an account of a new trypanosomiasis of man in Brazil, South America, which is transmitted by a biting bug (*Conorhinus megistus*), and of which the etiologic

factor, for reasons that will be apparent later, is named *Schizotrypanum cruzi*. This trypanosome is said not to multiply by simple division in the peripheral blood, but instead to undergo schizogony in the capillaries of the lungs of the infected animal. This reproductive process takes place at the time of the increase of the trypanosomes in the blood, especially at five to six days after inoculation of the animal and, also, during the great increase of the parasites which occurs just previous to the death of the infected animal. The trypanosome sheds its flagellum and undulating membrane, bends upon itself, and becomes fused into a round or oval body. In some of these bodies, the blepharoplast is shed with the flagellum, in others it is retained. By division of the nucleus in the first form and of the nucleus and blepharoplast in the second form, and by a differentiation of the protoplasm there are developed from these bodies schizocysts containing 8 small club-shaped merozoites. The merozoites escape from the cyst and penetrate red blood-corpuscles, where they develop into trypanosomes. When fully developed, they leave the blood-corpuscles and live free in the plasma. The blepharoplast-less merozoites develop into female trypanosomes, having a small blepharoplast derived from the nucleus through heteropolar division, a round nucleus, and a broad body; the merozoites having a blepharoplast develop into male trypanosomes having a large blepharoplast, an elongated nucleus, and a slender body.

Hartmann, in 1910, found in a section of the lung of a guinea pig infected with *Schizotrypanum cruzi* Chagas, greatly hypertrophied endothelial cells containing large numbers of more or less pyriform, binucleate bodies. Similar intracellular stages, Hartmann states, were subsequently found by Chagas in the heart musculature and in the brain of a man dead from schizotrypanosomiasis. He believes that this intracellular multiplication represents the true schizogony, while the extracellular schizogony with a sexual differentiation of the products of division, previously described by Chagas, is considered a gametogony. Carini (1911) has described a similar so-called schizogony of *Trypanosoma leptodactyli* in endothelial cells in the blood from the heart of an infected animal (*Leptodactylus ocellatus*).

Fantham (1911) has recently studied the life history of *Trypanosoma gambiense* and *Trypanosoma rhodesiense* in relation to the number of trypanosomes in the peripheral circulation, making use of the thick film method of Ross to determine accurately the number of trypanosomes in the blood from day to

day. He has confirmed the observations of Salvin-Moore and Breinl that small round bodies are developed in the lungs of the vertebrate host at or near the maximum of the multiplication of the trypanosomes in the circulating blood, and that these "latent bodies" persist in the spleen and bone marrow during the period when the trypanosomes are absent from the blood. However, Fantham does not agree with Salvin-Moore and Breinl as to the method of development of these bodies. He was unable to observe the interaction between the blepharoplast and nucleus of the trypanosome described by the latter authors. In the process as described by Fantham in the living trypanosome from the circulating blood under the microscope, the anterior end of the trypanosome disintegrates, the blepharoplast migrates near the nucleus and the posterior end of the trypanosome with the remnant of the flagellum is cast off. The round body, consisting of the nucleus and blepharoplast surrounded by a thin layer of protoplasm, constitutes the "latent body." Fantham was also able to observe the metamorphosis of the "latent bodies" into trypanosomes by mixing infected spleen pulp with fresh blood of an uninfected rat and observing it on a warm stage under the microscope. Finally, he claims to have demonstrated the infectiousness of this stage of the trypanosome by inoculating animals with spleen pulp found by microscopic examination to be free from motile trypanosomes but to contain the "latent bodies." Fantham, like Salvin-Moore and Breinl, is of the opinion that these bodies are a more resistant stage of the trypanosome and he believes that consideration should be taken of them and of the time of their development in the chemotherapeutic treatment of trypanosomiasis.

On the other hand, Laveran (1911), who has recently studied these round bodies of *Trypanosoma gambiense* in guinea pigs, has come to the conclusion that they are involution forms of the trypanosome, which are naturally very numerous in the spleen and bone marrow at the crisis of the infection.

Buchanan (1911) has observed some of the forms described by these other authors in the internal organs, bone marrow, and axillary glands of the gerbil (*Gerbillus pygargus*) infected with *Trypanosoma brucei* (*pecaudi*). This author describes and gives illustrations of round binucleate bodies found in smears from the lungs of animals killed on the sixth day of infection which correspond to the "latent bodies" of *Trypanosoma gambiense* and *Trypanosoma rhodesiense*. The development of these bodies, as observed by Buchanan, differs from that described

by either Salvin-Moore and Breinl or Fantham. The trypanosome bends upon itself ventrally and becomes fused into a round, binucleate body, around the border of which the flagellum remains for a time attached, but is later cast off. This method of development, therefore, corresponds more nearly with the formation of the schizonts of *Schizotrypanum cruzi*. In the spleen, bone marrow, and axillary glands round, ring, and coiled forms were found on the fourth to sixth day, usually surrounded by a clear area which gave the impression that the protoplasmic mass was lying in a vacuoloid space that was surrounded by a limiting membrane. In these forms the blepharoplast frequently appeared to lie detached from the protoplasmic mass in the clear area. Buchanan also found in smears of the spleen ring-form parasites and all stages in the development of these to the fairly mature trypanosomes within the red blood-corpuscles, which appear to be similar to the intracorpuscular stages of *Schizotrypanum cruzi* described by Chagas.

In the development of *Trypanosoma evansi* in guinea pigs, round nonflagellated forms are found in the spleen and bone marrow that correspond to the "latent bodies" described by Salvin-Moore and Breinl in *Trypanosoma gambiense* and by Fantham in *Trypanosoma gambiense* and *Trypanosoma rhodesiense*. These bodies of *Trypanosoma evansi*, however, appear to be not "latent," but developmental, forms which undergo a schizogony comparable to that taking place in *Schizotrypanum cruzi*. While all of the relations of this reproductive process to the life-cycle of the parasite have not been worked out, nor the process studied in the natural host of this trypanosome, it has seemed advisable to publish a preliminary account of it. The hope is expressed that it may lead to a more careful study of what appears to be early stages of the same reproductive process in *Trypanosoma gambiense* and other species of *Trypanosoma*, and to a further investigation of the relation, if any, of the different stages of this process to latency in trypanosomiasis and to relapses after chemotherapeutic treatment.

Two strains of *Trypanosoma evansi*, one from a horse and the other from a carabao, have been studied. These two strains have been propagated by subcutaneous inoculations from guinea pig to guinea pig, and the development herein described is based upon the study of the blood and organs of these animals.

The blood, taken from the ear veins, and the internal organs of guinea pigs killed at different periods of the infection, have been studied fresh, in dried smears, in smears fixed wet, and

in paraffine sections. These preparations have been stained with Giemsa's stain, aqueous alum hematoxylin, Mallory's ferric choloride hematoxylin, and Seidlin's iron hematoxylin. Air-dried smears of both the blood and organs, stained twelve to twenty-four hours with Giemsa's stain, have given the best results. The sections of the organs have been useful in determining the relation of the stages of development of the trypanosome to the tissues.

Trypanosoma evansi, when inoculated subcutaneously into guinea pigs, has an incubation period that varies from five to seventeen days, depending upon the strain of the virus, the number of trypanosomes inoculated, and the susceptibility of the animal. The disease runs a more or less chronic course in these animals, usually of from one to several months' duration. The course of the disease is marked by alternating phases of increase and decrease of the parasites in the peripheral blood. The trypanosomes will increase to a maximum and often swarm in the blood for several days; they will then decrease, and may wholly disappear from the peripheral circulation for several days, only to reappear and repeat the cycle. Sometimes one or more smaller crests of multiplication will intervene between two larger crests. Occasionally the infected guinea pig will die at the summit of the first crest of multiplication of the parasites in its blood.

Guinea pigs have been killed at different periods in the multiplication curve of the parasites in the blood and preparations from the different internal organs examined. It has been found, corresponding to the observations of Salvin-Moore and Breinl on *Trypanosoma gambiense* and of Fantham on *Trypanosoma gambiense* and *Trypanosoma rhodesiense*, that at or near the maximum of the increase of the trypanosomes in the blood large numbers of round, binucleate bodies are developed in certain of the internal organs. Guinea pigs inoculated intraperitoneally and killed from the fifth to eighth day, according to the directions of Chagas for finding the schizonts in *Schizotrypanum cruzi* and followed by Buchanan in studying the development of *Trypanosoma brucei* (*pecandi*) in the internal organs of the gerbil, showed only a few of the round forms. Guinea pigs, killed during the decrease or the absence of the trypanosomes from the peripheral blood, showed few or no round forms in the organs.

The place of development of these round forms of *Trypanosoma evansi* does not correspond to that of the development of the "latent bodies" *Trypanosoma gambiense* and *Trypanosoma*

rhodesiense or to that of the development of the schizonts of *Schizotrypanum cruzi*. According to Salvin-Moore and Breinl, Fantham, and Chagas the round forms of these trypanosomes are developed in the capillaries of the lungs of the infected animals. In *Trypanosoma evansi*, on the other hand, the round forms are developed chiefly in the spleen and to a lesser extent in the bone marrow. A few of them can sometimes be found in the lungs and other internal organs, where they have probably been carried by the circulating blood. This corresponds to the observations of Buchanan on *Trypanosoma brucei*. The spleen is always more or less enlarged, congested, and dark in color. Sections of the organ show that the round forms are developed extracellularly in the small capillaries, which are often occluded by them.

Furthermore, the development of these round forms of *Trypanosoma evansi* is different from that described in *Trypanosoma gambiense* and *Trypanosoma rhodesiense*. Salvin-Moore and Breinl and also Fantham disagree as to the details of the development of these bodies, but both agree that a large part of the trypanosome degenerates and is cast off, and that the round body consists only of the nucleus, blepharoplast, and a small remnant of the protoplasm of the trypanosome. According to my observations the round form of *Trypanosoma evansi* is made up of the whole trypanosome, with the exception of the flagellum which is cast off. The trypanosome bends upon itself ventrally (Plate I, fig. 2) until the anterior and posterior ends are apposed, the halves then fuse to form a round or oval body, around the border of which the flagellum remains for a time attached (fig. 3). Sometimes by the fusion of the two ends of the coiled-up trypanosome, ring-shaped bodies are developed which later become fused into a solid mass. The flagellum attached about the border of the rounded trypanosome soon becomes detached, leaving the nonflagellated, binucleate body. Therefore, the development of these bodies in *Trypanosoma evansi* corresponds more nearly to the development of the forms described by Buchanan in *Trypanosoma brucei* and to the development of the schizonts described by Chagas in *Schizotrypanum cruzi*.

These bodies in *Trypanosoma evansi* (fig. 4) are round or oval, 2 to 5 microns in diameter, stain pale blue, and contain a nucleus and a blepharoplast that stain red with Giemsa's stain. The nucleus may be situated centrally, but more often eccentrically. The blepharoplast is usually eccentrically placed, often at the side opposite to the nucleus; sometimes it is adjacent to

the nucleus. They bear a resemblance to the Leishman-Donovan bodies. I have not been able to distinguish a blepharoplast-less variety corresponding to the female type of schizont described by Chagas in *Schizotrypanum cruzi*. Sometimes the blepharoplast lies closely applied to or over the nucleus, where it is distinguished with difficulty. These bodies do not appear to be surrounded by a definite wall or limiting membrane, but are probably bounded by the periplast of the trypanosome.

In the spleen of guinea pigs killed when the blood is swarming with trypanosomes, a further development of these bodies is evident. The round binucleated body increases in size, and concomitantly a multiplication of both nucleus and blepharoplast takes place. The first evidence of these nuclear changes is seen in forms like that shown in fig. 5 which contains one nucleus but in which the blepharoplast has divided. Succeeding stages containing 2, 4, 6, and 8 nuclei, and blepharoplasts are shown in figs. 6 to 10. The fully developed schizonts are round in optical section and measure 10 to 15 microns in diameter. The average number of nuclei and blepharoplasts appear to be 8, but the number varies from 4 to 16. Some of the large schizonts (fig. 11) show evidence of fission of the protoplasm, and others (fig. 12) show a complete division and differentiation of the merozoites, surrounded by a thin cyst wall. In schizocysts that are not ruptured or deformed the merozoites appear to be arranged like the segments of an orange, but with a slight spiral twist. The merozoites are elongated, sickle-shaped bodies, 6 to 10 microns long and 1 to 1.5 microns broad, are without undulating membrane and flagellum, and have a nucleus situated near the center and a blepharoplast near one end.

The merozoites of *Schizotrypanum cruzi*, according to Chagas, escape from the cyst and penetrate red blood-corpuscles of the host where they develop into adult trypanosomes. Buchanan, also, observed ring-form, binucleate parasites and all stages of the development of these to adult trypanosomes in the red blood-corpuscles from the spleen of the gerbil infected with *Trypanosoma brucei*. Forms, corresponding to these intra-corpuscular stages of Chagas and Buchanan, are frequently seen in the smears from the spleen of guinea pigs infected with *Trypanosoma evansi*, but in no case have I been able to convince myself that the parasite lay within the red corpuscle. In spreading a smear of the spleen pulp containing many trypanosomes it must frequently happen that a parasite will lie over, under, or around a red corpuscle; and in drying such a smear the

parasite may be pressed more or less into the surface of the plastic corpuscle and appear as if intracorpuscular in the stained preparation. The merozoites of *Trypanosoma evansi,* so far as my observations show, develop extracorpuscularly and directly into adult trypanosomes.

No sexual process has been observed preceeding or during the formation of the round bodies from the trypanosomes, nor during the development of the multinuclear cysts. Moreover, the sexual reproduction of trypanosomes should, according to the accepted theory, take place in the invertebrate host. The merozoites of *Trypanosoma evansi* do not show the dimorphism described by Chagas in *Schizotrypanum cruzi,* nor have I observed any evidence of a sexual differentiation in the adult trypanosomes. Therefore, I shall provisionally designate this reproductive process in *Trypanosoma evansi* as a schizogony. The occurrence of the young schizont (round or "latent") stage in *Trypanosoma gambiense, Trypanosoma rhodesiense, Trypanosoma brucei* (*pecaudi*), and *Trypanosoma leptodactyli* indicates that this schizogony is a general reproductive process in the *Trypanosomata.*

The genus *Schizotrypanum* established by Chagas for the parasite of South American trypanosomiasis of man may have to be abandoned. Its chief differential character from the genus *Trypanosoma* appears no longer to exist. The so-called intracorpuscular stages of *Schizotrypanum cruzi* have been observed, also, in *Trypanosoma brucei* and in *Trypanosoma evansi.* In the last species the parasites, although apparently within the corpuscle, are really extracorpuscular. The schizogony of *Schizotrypanum cruzi* described by Hartmann in the endothelial cells of the lung has been observed in *Trypanosoma leptodactyli* by Carine in endothelial cells in the blood from the heart of an infected animal. This so-called schizogony consists in both cases of hypertrophied endothelial cells containing a large number of the round or "latent" forms of the trypanosome. It appears to be a case of phagocytosis of the young schizonts by an endothelial macrophage. Phagocytosis of the trypanosomes, of the round forms, and occasionally of the large schizonts by the macrophages appears to be a common phenomenon in trypanosomiasis; indeed, this is the fate of most of the trypanosomes when they disappear from the blood in the latent phase of the disease. The absence of multiplication by simple division in the peripheral blood would appear to be distinctive of *Schizotrypanum cruzi;* but, in view of the fact that this parasite appears to

multiply by simple division in the gut of the invertebrate host (*Conorhinus megistus*) and in cultures on Novy and McNeal's medium, a suspicion is raised of the accuracy of the observation that it is absent in the peripheral blood of the vertebrate host.

The significance of this schizogony in the life-cycle of the trypanosome is uncertain. Fantham as well as Salvin-Moore and Breinl consider the round form of *Trypanosoma gambiense* and *Trypanosoma rhodesiense* to be a resistant stage of the parasite which persists during the latent phase of trypanosomiasis when the parasites are absent from the peripheral blood. Salvin-Moore and Breinl designate them as such by the name "latent bodies," and Fantham suggests, although he does not definitely state, that these latent bodies may be resistant to drugs used in the treatment of trypanosomiasis and may be responsible for the relapses that occur after chemotherapeutic treatment. If these so-called latent bodies are, as my observations have indicated, only an early stage in the development of the schizonts, it would seem more probable that the mature schizocysts might be the resistant stage of the trypanosome. However, an examination of the spleen and other internal organs of guinea pigs killed during the decrease of the trypanosomes in the blood, or during the latent phase of the infection, has shown no schizocysts but only a very few flagellated trypanosomes and round forms (schizonts). It is possible, therefore, that some of the young schizonts persist through the latent period and undergo schizogony at the beginning of the relapse, giving rise to the new generation of trypanosomes. It is also possible that it is unnecessary to assume the existence of a special resistant stage of the trypanosome to account for latency and relapse in trypanosomiasis. Further morphological and experimental investigation is necessary to decide those questions.

CONCLUSIONS.

In the developmental cycle of *Trypanosoma evansi* a schizogony takes place in the spleen of the vertebrate host.

The observations of Salvin-Moore and Breinl, Fantham, and Buchanan that forms similar to the young schizonts of *Trypanosoma evansi* occur in the internal organs of animals infected with *Trypanosoma gambiense*, *T. rhodesiense*, and *T. brucei* make it probable that schizogony is a reproductive process common to the trypanosomata.

The validity of *Schizotrypanum* Chagas as a genus distinct from *Trypanosoma* Gruby appears to be doubtful.

Further investigation is necessary to determine the significance of this schizogony in the life-cycle of the trypanosomata and its relation, if any, to latency in trypanosomiasis and to relapses after chemotherapeutic treatment.

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ILLUSTRATIONS.

(From water-color drawings by Teodosio S. Espinosa.)

PLATE I.

The figures in Plate I were drawn with the camera lucida at a uniform magnification of 2,000 diameters.

FIG. 1. Motile form of *Trypanosoma evansi* from the blood of an infected guinea pig.

2. A trypanosome coiling up to form a schizont ("latent body"), from the spleen of an infected guinea pig.

3. A schizont ("latent body") with the flagellum still attached, from the spleen of an infected guinea pig.

4. A schizont ("latent body") of *Trypanosoma evansi*, from the spleen of an infected guinea pig.

FIGS. 5 to 10. Schizonts showing successive stages of division of the blepharoplast and nucleus, from the spleen of an infected guinea pig.

FIG. 11. A schizont showing the beginning of the formation of the merozoites, from the spleen of an infected guinea pig.

12. A mature schizocyst containing 8 merozoites (semidiagrammatic), from the spleen of an infected guinea pig.

FORMS OF TRYPANOSOMA EVANS

PLATE I

REVIEW.

Pathology and Bacteriology of the Eye. An International System of Ophthalmic Practice. By E. Treacher Collins, F. R. C. S., and M. Stephen Mayon, F. R. C. S. Edited by Walter L. Pyle, A. M., M. D., Philadelphia, Pa.

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Held at Mukden, April, 1911, under the auspices of the Chinese Government.

Edited by ERICH MARTINI, G. F. PETRIE, ARTHUR STANLEY, AND RICHARD P. STRONG.

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The Bureau of Science of the Government of the Philippine Islands has been appointed sole agent for the distribution of the printed proceedings of the International Plague Conference.

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146 pages, 10 plates, 1 map.

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